STANFORD STUDIES
IN THE MEDICAL SCIENCES VIII

BIOCHEMICAL CONTRIBUTIONS TO ENDOCRINOLOGY

The Lane Medical Lectures, 1956

BIOCHEMICAL CONTRIBUTIONS TO ENDOCRINOLOGY

EXPERIMENTS IN HORMONAL RESEARCH

BY SIR CHARLES DODDS, M V O

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PREFACE

The following five papers — the Lane Medical Lectures of 1956 — were delivered in their original form last autumn at the Medical School of Stanford University in San Francisco They have been revised for publication and I should like to thank the Stanford University Press for arranging for their appearance in book form

To be invited to lecture at an American university is a compliment and if that university be Stanford then it is a distinction but if Stanford University appoints one to the Lane lectureship, then that is indeed a very great honor I must commence by expressing my very deep appreciation of having been invited to give this series of lectures and I must also mention my awareness of the great responsibility that went with the honor

To Professor Windsor C Cutting I owe a special debt of gratitude for the careful preparation made by him in arranging for the lectures It was also a pleasure to discuss, with him and with his colleagues at San Francisco and at Stanford, the subject of the lectures both before and after they were delivered

I also wish to acknowledge the help of my colleague, Dr E T Knudsen in putting the lectures in a form suitable for publication and I should like to thank Mrs E I Barron, who has played a major part in the preparation of the manuscript

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May 1 1957

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BIOCHEMICAL CONTRIBUTIONS TO ENDOCRINOLOGY

I INTRODUCTION TO BIOCHEMICAL RESEARCH IN ENDOCRINOLOGY

I have chosen my title of "Biochemical Contributions to Endo crinology because it is one that covers almost completely the work of myself and my colleagues in the Courtauld Institute of Bio chemistry during the last thirty odd years Much of this work has been published a good many years ago but it occurred to me that it would perhaps be of interest were I to say in greater detail how the work developed No doubt a great deal can be gained by recounting the mistakes one has made and the attempts to rectify them which lead very frequently in directions quite differ ent from those in which one originally started

We may perhaps commence with some general observations of modern research in relation to therapeutics Today we know that scientific research in medical and allied subjects is constantly scrutinized with a view to its application to therapy and there is a feeling that this has always been so It is in fact a very modern trend and certainly this attitude developed only within the last hundred years While the nineteenth century can definitely be described as the golden age of surgery we have to wait until the twentieth century before we can say that modern medicine really began Medical historians and particularly those interested in the scientific aspect tell us that modern medicine began with Har vey s description of the circulation in the seventeenth century While this may be true from a historical point of view, it is cer tainly not true from the practical angle If one reads Harvey s publications carefully, one can see quite clearly that his great discoveries were not in any way connected in his mind with ther apy Despite the descriptions in the famous work *Exercitatio ana tomica de motu cordis et sanguinis* (24) the Galenical concept of the four humors—blood phlegm, yellow and black bile—still

dominated the minds of physicians and was not finally disposed of until the end of the eighteenth century and the beginning of the nineteenth

Of course, one of the great handicaps of the earlier physicians was their lack of really powerful drugs The great pharmacopoeias prepared by the Royal College of Physicians, dating from 1618, contained what we now know to be practically worthless drugs Apart from opium, quinine, and, later, digitalis there were really no effective therapeutic agents whatsoever, and we can therefore see, if we study the treatment of a disease such as pneumonia throughout the sixteenth seventeenth eighteenth, and nineteenth centuries, that treatment altered little A study of the treatment of pneumonia in the seventeenth, eighteenth, and nineteenth centuries reveals that the basic treatment consisted of blistering bleeding scarification, dry-cupping, and, in some cases, enemas or clysters In reading the early physicians' accounts of the treatment one cannot help wondering whether people would not have been better off without any physicians at all The difficulty, of course, in assessing this point is that usually one has no controls, but fortunately in the case of pneumonia there is a perfect series of controls for treatment of this condition in the eighteenth and nineteenth centuries Toward the end of the eighteenth century and the beginning of the nineteenth, the strange figure of Hahnemann (1755–1843) dominated the medical therapeutic world of Europe (23) We shall not now enter into a discussion of his fantastic theory of potentiation by dilution, which was the basis of homeopathy, and we now know that practically the whole series of drugs in his pharmacopoeia were without activity even if given in much greater doses than the minute ones employed by his system The strange fact was that his patients in many cases did very much better than those treated by the members of official bodies such as the College of Physicians In fact so successful was he that he retired to Paris in the 1830 s a millionaire having made it all out of his therapy We now know that his success was really the result of doing nothing and that to use an old fashioned expression he had given nature a chance One should never lose sight of the lesson provided by this study, and I should like to quote a very witty summary of the situation given in Allbutt and Rolleston s *System of Medicine* (1)

It is a humiliating but instructive fact that the possibility of recovery from acute disease without active treatment was e tablished by the assumed success of a demonstrably futile system of therapeutics the last, we may hope of attempts to answer the absurd question "On what universal principle should disease be treated?" When it could not be denied that persons suffering from pneumonia and other acute disorders did recover when treated with infinite imal doses of useless drugs it could not be long doubted that some acute diseases might get well of themselves

The report of some ca es of pneumonia which recovered in the Homeopathic Hospital at Vienna awakened thought on this subject, and an article by Sir John Forbes which appeared in the British and Foreign Medico-Chirurgical Review [22] pressed the lesson home Skoda [32] had given fair trial to other methods of treatment and found that under the o-called expectant treatment the mortality of his patients from acute pneumonia was much less than when treated by bleeding blisters and antimony These facts were made known in England by George Balfour [2] who had fol lowed Skoda s practice in Vienna John Hughes Bennett of Edinburgh also published a series of cases of pneumonia treated without bleeding anti mony or mercury with unusually small mortality [3] and he gave an interesting account of the arguments of Alison Watson, Christison and Markham Di cussion followed but it was less prolonged than might have been supposed as so often happens general opinion had been gradually altering and was ready to turn at the first summons Moreover the advocates of antiphlogistic treatment threw away their case by the assertion that they were right in bleeding before, and right in doing nothing after wards—not because their opinions but the nature of the disease had changed and a presumed sthenic type of fevers and inflammations, with a succe sful heroic treatment corresponding thereto was dwelt upon with the same satisfaction that an old man contrasts the hard frosts and heroic exploits of his youth with the mild winters and feeble powers of his con temporaries.

Pasteur s demonstration of the microbial origin of disease (30) together with the development of synthetic organic chem istry toward the latter half of the nineteenth century changed the whole picture and we had the introduction of the first chemothera peutic agent by Ehrlich in 1910 (21) From this period onward the eyes of therapeutists were firmly fixed on the laboratory and we find the sulphonamides in the 1930 s very quickly establishing their position while the antibiotics were translated straight into medicine from the moment of their production From the work that I am about to describe we shall be able to see that substances produced in our laboratory such as the synthetic estrogens very quickly found a place in therapy and the discovery of new sub-

stances secreted by the body, such as aldosterone, very quickly began to modify and explain a number of conceptions of disease

Substances affecting oxidation in the human body

Cutting Mehrtens, and Tainter (6) described in detail the remarkable effects on metabolism of administering 2 4 dinitro phenol It was shown that by the oral administration of daily doses of 3 mg per kg body weight of 2 4-dinitrophenol a marked stimulation in metabolism could be obtained and this was followed, provided the diet remained the same, by a rapid loss of weight The observation that 2 4 dinitrophenol produces a stimulation of metabolism goes back a considerable way, as it was first observed in workers involved in the preparation of this substance for explosive purposes Cutting and his collaborators pointed out that 2 4-dinitrophenol was a toxic substance quite apart from its power to raise the basal metabolic rate and that, therefore its clinical use had to be very carefully guarded Sir William Pope and I were engaged in the study of the biological effects of substituted phenols at this time, and we were very interested in comparing the activity of dinitro ortho cresol with that of 2 4 dinitrophenol The acute toxicity of both these substances was about the same, but we were able to show by animal experiments that the dinitro ortho cresol was considerably more powerful than the 2 4-dinitrophenol, although, of course the mechanism of stimulation of oxidation was undoubtedly the same (13) We now know that all substances of this type are too dangerous to be used clinically for a number of reasons Above all by their very powerful nature they are themselves extremely dangerous, for unless administration is continued by very frequent estimations of basal metabolic rate severe hyperthermia and death may result It is interesting to note that it is possible to increase the basal metabolic rate by nearly 100 per cent without any corresponding alteration in the circulation rate The only method apart from the determination of basal metabolic rate of following the change is to determine the oxygen saturation of the venous blood This would be found to be very much diminished

The appearance of bilateral cataracts in a number of patients who had used this type of drug for reducing purposes at once put an end to the therapy and we are, therefore, only interested in

this group from a purely theoretical point of view J D Robert son and I were however able to use dinitro ortho cresol to settle the real question as to whether myxedema was due solely to a re duction in the basal metabolic rate (14) Figure 1 shows a myxe dematous man whose basal metabolic rate has been raised to plus 20 per cent by dinitro ortho cresol The second picture shows the same patient with a basal metabolic rate of plus 10 per cent pro duced by the administration of thyroid extract In the latter case it can be seen that the signs of myxedema have disappeared while in the former, despite the fact that the basal metabolic rate is higher, the stigmata of the disease are very clearly present This adequately confirms the conception that thyroid hormone has a very specific action quite apart from the mere raising of the meta bolic rate A study of the effect of these polyphenols on the metabo lism of tissue slices, as studied by the Warburg technique has pro vided a series of researches Some of the early work was done by me and my colleague, *G D Greville* and we were able to show that unlike other stimulators of metabolism the poly nitro phenols stimulated both respiration and glycolysis This occurred in nor mal and in tumor tissue (12 15) During the 20 years that have elapsed since these results were published the whole question of tissue metaboli m and oxidation has made very great advances but recent work on the action of the poly nitro phenols has emphasized the correctness of our original view namely that they act as a general stimulus to the action of all the oxidative systems in the tissue

The pituitary and the control of gastric secretion

In the year 1924 my colleague F Dickens and I were inter ested in studying methods for the production of insulin The stand ard technique of the day was the alcohol process worked out by the original Toronto research workers We had been interested in studying the properties of insulin picrate and we were able to show that it was differentially soluble in a 70 per cent aqueous ace tone solution We therefore developed a process (10 11) in which picric acid was applied direct to the mixed pancreas, and the insulin picrate was extracted with 70 per cent aqueous acetone The hydrochloride was regenerated from the extracted insulin picrate and it was found that the yield was very high indeed This method

was used successfully in commercial production of insulin, but in the long run modifications of the alcohol processes proved to be more efficient and in any case produced a substance capable of much greater purification so that the acetone–picric acid process as it was called, became redundant and was only used for the estimation of insulin content of tissues

In the early 1930's there was a great awakening of interest in pituitary hormones, and it was decided in my laboratory to apply the acetone–picric acid process to the pituitary to see what effects could be obtained from extracts prepared by this method The original process was applied to pituitary glands obtained from the slaughterhouse and a good yield of hydrochloride was obtained This substance was readily soluble in water and was a white non deliquescent powder Its properties were described by Noble, Smith, and myself in a paper (16) We investigated the general effects of this hydrochloride when administered to laboratory animals (18) Our first experiments were conducted on rabbits of 2 to 3 kg weight, and an injection of 150 mg of the material was arbitrarily chosen as a suitable commencing dose It was quite obvious that the effect on the animals was marked The first find was severe prostration from which the animal recovered in a few hours Most of the animals recovered, but we noticed that they ate nothing for some seven to ten days and the feces contained altered blood After this period the rabbits ate voraciously and regained the weight they had lost very rapidly becoming normal It was apparent that something very dramatic must have taken place in the alimentary canal to produce these marked changes

We decided to investigate the alimentary canal when we imagined the lesion, if any would be at its height, some fifteen to twenty *four hours after the injection* On opening the abdomen of the rabbit killed under these conditions it was immediately seen that there was a marked change in the stomach The fundus as seen from without, was plum-colored and was obviously the seat of a severe lesion On removing the stomach and washing out the food residue a most remarkable lesion was evident The esophagus duodenum and distal portion of the stomach appeared the normal pale color which we associated with the mucous membrane in this region The fundus, however presented a dark red mottled appearance with a fibrinous exudate on the surface (Fig 2)



Basal metabolic rate ra sed to plus 20 per cent by din tro ortho cresol Myxedema unrelieved

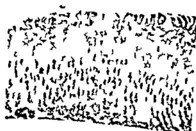


Basal metabolic rate raised to plus 10 per cent by thyroid Myxe dema completely relieved

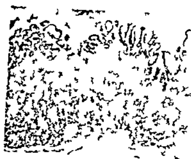
Figure 1



Figure 2



F gu e 3



Figu e 4



F gu e 5



Figu e 6

Marked edema of the walls of the stomach beneath the mucosa usually accompanied the lesion in fact the whole appearance was very similar to that seen in the post mortem examination of human subjects who have taken a large dose of some severe corrosive poison

This experiment was repeated over fifty times and the result was always the same namely a severe lesion confined entirely to the acid bearing area of the stomach A careful examination of the other organs of the body showed that they were unaffected The problem arose therefore as to the nature of the mechanism producing this lesion whether it was a pharmacological effect or whether it represented the exaggeration of some naturally operating control of gastric secretion from the pituitary body

It was at this stage of our investigations that we were fortunate in being joined by Dr Windsor Cutting who spent some years in our laboratory and was present during the unraveling of this extremely complicated story The following account is based on a series of publications in the *Proceedings* of the Royal Society appearing in 1937 (7 8 9 19) One of the first questions to arise concerned the nature of the gastrotoxic factor Experiments very quickly showed that the toxic factor was confined to the posterior lobe of the pituitary since extracts of the anterior lobe had no effect whatsoever Careful investigation of the oxytocic and vasopressive fraction showed that the former was without action, while the whole effects of our acetone–picric acid process extract could be obtained by the pituitary preparation containing only the vasopressive substance We therefore concluded that our toxic factor was either the vasopressive itself or a substance associated with it Investigations in recent years have shown that the action is given by the pure vasopressive substance therefore we must now conclude that the results originally described by us in 1934 were due to the administration of an excess of vasopressive factor

The lesion can be produced no matter how the vasopressive factor is introduced whether by subcutaneous intravenous or intracisternal injection Provided the pituitary extract is given in the form of the standard acetone–picric acid preparation it will also produce the lesion when given by mouth With regard to the type of animal we were able to show that typical lesions could be produced in the monkey cat rabbit guinea pig rat and mouse

These results have been confirmed by a number of other workers (4 26), and lesions have also been shown to be present in the dog (27) when the extract is given in adequate dose As I have already stated, in all animals it is found that the lesion is con fined to that area of the stomach in which oxyntic cells are present If the extract is administered to an animal undergoing an acute experiment in which the stomach is exposed a very interesting series of changes can be seen Injection of the material intra venously produces a blanching of the entire stomach mucosa This is followed about an hour later by marked dilatation and en gorgement of the capillaries Shortly afterward exudation and hemorrhage appear A careful histological examination of sections taken during this period confirms microscopically the dilation Figures 3, 4 and 5 show the microscopic appearance one hour, three hours, and six hours respectively after injection of pitui tary extract (x60) Very early changes occur in the oxyntic cells, and blurring of the cell outline and pyknosis of the nuclei are common As already stated there is no sign of a lesion anywhere else in the mucosa of the stomach esophagus or duodenum Only in one instance did we find an isolated lesion in the pylorus and on section this was found to be an area of isolated rest of oxyntic cells In the rabbit we found occasionally punched out ulcers—in one case definitely showing a perforation sealed by a piece of omentum (Fig 6) The interesting point is that even after so severe a lesion complete healing can occur and the animal will show no change whatsoever

Attempts to imitate the action of the pituitary extract with other drugs proved to be unsuccessful, with one exception While atropine pilocarpine adrenalin and histamine in the largest doses possible have never shown any lesions, the administration of normal hydrochloric acid into the stomach of the anesthetized ani mal produced a typical lesion The injection of intense vasocon strictors such as barium chloride produced a typical lesion and therefore we concluded that an essential part of the mechanism was a diminution in the blood supply caused by vasoconstriction

As previously *stated other organs of the injected animals* appeared to be unaffected We were however able to demon strate another peculiar finding A number of animals injected with the extract showed a very severe anemia This after all is

not surprising when one considers the extensive nature of the lesion and the persistence of melena for some days after the injection Together with Noble (17) I made a detailed study of the anemia This appears on the fourth to fifth day and the red blood cell count may be reduced to as low a figure as a million red cells per cu mm This is associated with a leucocytosis of up to 50 000 white cells per cu mm Hemoglobin falls considerably lower than the diminution of the red blood cell count would lead one to expect Reticulocyte response usually sets in on the fifth to sixth day and continues up to the eighth Reticulocyte counts as high as up to 50 per cent were observed A study of a stained smear shows a very interesting series of changes The cells appear to be well filled with hemoglobin but anisocytosis is present to a very marked extent Large numbers of macrocytes and a few microcytes are seen in the smear Also nucleated red cells have appeared It is interesting to note that one cannot produce this type of change by experimental hemorrhage The whole picture is very similar to that seen in pernicious anemia of the human subject the anemia being very definitely of the macrocytic variety A return to this subject was made during the war and it was shown that these changes occur in some 20 per cent of animals injected Owing to the difficulty of obtaining stock at this period these experiments were not continued but they certainly raise the interesting question as to whether there is some centralized control of hemopoiesis

Effect of posterior pituitary extract on gastric and intestinal secretion

Experiments were made mainly on rabbits and cats These were anesthetized with nembutal and a glass cannula was stitched into the most dependent part of the stomach the pylorus being tied off In the cats used for the chronic and prolonged experiments a permanent external gastric fistula had been previously created and it was found that with training it was possible to collect gastric juice in a pure form after the stomach had been emptied The effect of various stimuli on gastric secretion was studied It was found that histamine was one of the most suitable In order to obtain a more physiological reaction we also studied the effect of the administration of insulin It had previously been established (28 31 25 5) that the production of a sudden hypogly

cemia would cause a marked secretion of gastric juice This we were able to confirm in the cats We were able to show that gastric secretion, produced by whatever cause, is immediately abolished by the introduction into the circulation of an adequate quantity of the vasopressive hormone of the posterior lobe (8)

In a further series of experiments we were able to show that the lesion developed as the result of cutting off the blood flow (9) We can therefore, see that the vasopressive extract of the pos terior lobe acts by reducing the blood flow to the stomach If the blood flow is reduced, then secretion is abolished We were also able to show that any other treatment that reduced the blood flow would at the same time inhibit the secretion, so we had to decide at this time whether this mechanism has any real physiological basis or whether it is only an interesting pharmacological observa tion To investigate this vital point we decided to study the gastric secretion in hypophysectomized animals (9) We used cats for the chronic experimentation, the animals were prepared with the same type of fistula as described for the work on the effect of agents on gastric secretion The hypophysectomy was performed by the buccal route under strict aseptic conditions using ether or nembutal as an anesthetic The animals required very careful postoperative treatment being extremely sensitive to changes in temperature Experiments on gastric secretion were then carried out, and when each experiment was completed the animal was killed and a very careful post mortem examination was conducted to make absolutely certain that the whole of the pituitary had been removed The results are described only on animals that obeyed this criterion Repeating the experiments with histamine and in sulin we were able to show very quickly that there is a marked difference between the animals with and without their pituitary This alteration is shown very clearly in Figures 7 and 8 Figure 7 shows the relationship of acid content to volume of gastric juice at various stages of the secretion It can be seen that there is a very clear relationship as the volume increases so does the acidity this is on normal intact animals Figure 8 shows the same ob servation on hypophysectomized animals it demonstrates very clearly that this important relationship between volume and acid ity has been lost

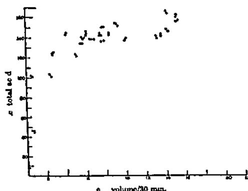


Figure 7 —Normal elat onship of acid to volume of gastr c ju ce H stam ne st mulus

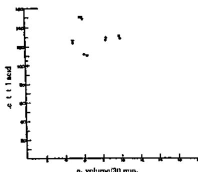


Figure 8 —Relationsh p of ac d to volume of g str c ju ce follow ng hypophysectomy H stam ne st mulus

General discussion

The investigations described in these papers arose from the observation that injecting into the rabbit an extract of the posterior lobe of the pituitary gland was capable of producing a severe lesion in the acid bearing area of the stomach In the earlier stages the experiments were designed solely to discover the cause of this lesion but it soon became apparent that the role of the posterior lobe of the pituitary body was even more complex than had originally been suspected and that its secretion exerted a controlling influence on glandular activity throughout the body The problem

was attacked by the administration of an excess of the extract and by a study of animals from which the gland had been removed

The administration of the extract to normal intact animals leads to a series of definite and consistent changes in the nature of their glandular secretion In the case of the stomach there is first of all a partial inhibition of the gastric secretion resulting in a reduction in the amount of juice produced The composition of this secretion does not vary much and the concentration of acid is usually unaffected But the total quantity or volume per unit of time is reduced If still more of the extract is administered then complete inhibition occurs and it is impossible to detect the secretion of any fluid by the stomach If the contents of the stomach in which secretion has been inhibited by this means are strongly acid, then a severe destruction of the gastric mucosa occurs with the production of the typical hemorrhagic lesion The lesion may heal perfectly or it may heal irregularly and produce a chronic lesion taking the form of an ulcer and subacute or chronic perforation of the stomach may result Experimental inquiry has established that two factors are necessary for the production of this gastric lesion inhibition of secretion and the presence of free acid in the stomach It would appear reasonable to assume that the stomach wall can only be protected from destruction by the continuous secretion of gastric juice, and that when this ceases the highly digestive fluid in the stomach is able to penetrate the mucosa and exert its destructive action It is generally accepted that the constituents of gastric juice are secreted by different cells of the stomach mucosa thus the hydrochloric acid would appear to be produced by the oxyntic cells while the other constituents appear to come from the chief cells throughout the stomach It will be remembered that the lesion only occurs in the area occupied by oxyntic cells and since it has been demonstrated experimentally that only the volume of juice is inhibited and not the acid secretion it would appear reasonable to suggest that in the normal animal the fluid provided by the continuous secretion is responsible for the removal of the hydrochloric acid which is secreted by the oxyntic cells The administration of an excess of pituitary extract stops this protective normal secretion of fluid and a lesion results

The mechanism of this inhibition has been fully investigated and the cau e shown to be a reduction in the blood flow to the

stomach Careful analysis of the phenomenon of gastric secretion has indicated that two factors are necessary an adequate stimulus and the ability of the vascular system to allow for a concomitant increase in the blood flow It has been shown that one without the other is not sufficient to produce a secretion In the normal animal there is a parallel relationship between the blood flow and secretion the two factors rising and falling together

Turning to the second method of investigation, a study of animals from which the gland has been removed it would appear to the casual observer that there is little or no alteration in the stomach Certain fine histological changes were observed and reported in the first paper, and certain gross lesions occasionally occur, but these will be referred to later A careful study of gastric secretion in the hypophysectomized animal reveals at once a fundamental difference from the normal In the first place, the regular relationship between blood flow and secretion is abolished and consequently the regular smooth relationship between acid and volume of gastric juice disappears In the normal animal a rise in volume is associated with an increase in acidity, and vice versa, but, as pointed out earlier in this discussion the administration of an excess of posterior lobe extract cuts down the volume and leaves the acid unaltered In the hypophysectomized animal this acid volume relationship is abolished, with the result that it is possible to find a low acid content with a high volume and the reverse Experiments have shown that the explanation lies in the fact that in the hypophysectomized animal the vascular system of the stomach is not capable of allowing a regular increase in blood flow following the application of a stimulus, and what might be termed a disorderly secretion results

In a few animals it is possible to see small hemorrhagic erosions in the acid bearing area of the stomach Parkes, in a personal communication (29) reported that in the ferret perforating ulcers have been encountered in a few hypophysectomized animals Macroscopically the lesion in the hypophysectomized animal presents an appearance similar to that produced by the administration of excess of the extract No lesion however, has ever been seen that approached in size and destructiveness that described in the injected animals While other possible causes are discussed in another paper (7) a probable explanation for these lesions in the

hypophysectomized animal may be that since the regular relationship between acid and volume is destroyed a local condition might occur in which acid was secreted without an adequate amount of fluid to remove it

In conclusion these investigations indicate that the posterior lobe of the pituitary gland produces a substance which up to the present has not been dissociated chemically from the vasopressive principle and which is necessary for maintaining the vascular system in such a state that it is capable of a smooth and regular activity, so that the blood flow to secreting glands such as the stomach may be coordinated with the secretion, following the application of an adequate stimulus

These findings demonstrate a new relationship between the posterior lobe of the pituitary, the blood flow and alimentary secretion, and they suggest an entirely new approach to research on diseases in which there is a derangement of the alimentary function

Other evidence of a dependence of gastric secretion on central nervous system control

For nearly 150 years pathologists have noticed an occasional relationship between intracranial lesions and pathological conditions of the stomach This subject has been very fully reviewed by Doig and Shafar (20) They note the number of references to cerebral conditions in which hematemesis and melena have been reported

Intracranial neoplasma	22	Intracranial aneurysm	1
Meningitis	8	Encephalitis	1
Head injury	5	Choreo athetosis	1
Bulbar poliomyelitis	*8*	*Cerebellar cyst*	*1*
Intracranial hemorrhage of the newborn	6	Following frontal lobotomy	2
Cerebral infarction	4	Following pneumoencephalography	2

The authors describe in detail seven cases of gastrointestinal hemorrhage associated with a cerebrovascular accident Both the macroscopic and microscopic appearances of the gastric lesions are described and possible mechanisms for the production of the gastric lesions are discussed

The histological appearance of many of the lesions described by Doig and Shafar is very similar to those in our experimental animals and it may well be that this is a further confirmation of the pituitary control of gastric secretion These authors however, do not report any examination of the pituitary

It is difficult on the evidence available at present to estimate the importance of the posterior pituitary control in relation to gastric secretion After discussions with others in this field and after studying the reviews of this work many authors have suggested that the vasopressive control of gastric secretion is not in effect a physiological mechanism, and that the results demonstrated by us fall more into the domain of experimental pharmacology and physiology Against this view I would strongly press the experiments on the hypophysectomized animals These results demonstrate quite clearly that there is a profound alteration in gastric secretion in these animals It is obvious that much more work will have to be done before these results can be satisfactorily interpreted We are now turning to this problem and hope in the future to establish on a direct experimental basis the important physiological effect of the vasopressive fraction of the posterior lobe In Europe there is a wave of enthusiasm for total hypophysectomy for various conditions in the human subject I am taking advantage of this by having the gastric secretion of the patients investigated before and after hypophysectomy and I hope we shall be able to report the findings at a later date

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The authors describe in detail seven cases of gastrointestinal hemorrhage associated with a cerebrovascular accident Both the macroscopic and microscopic appearances of the gastric lesions are described and possible mechanisms for the production of the gastric lesions are discussed

store the characteristic cyclical changes of estrus as judged by the vaginal smear, by the injection of an oily substance extracted from the ovary by means of volatile solvents This extract was stable and could be standardized with fairly good reproducibility, which undoubtedly formed the basis for our knowledge of the estrus producing hormone Their results were very rapidly confirmed and papers began to appear all over the world on the biological properties of this material By its use in the rat and mouse all the characteristic histological features of estrus could be made to reappear after bilateral oophorectomy While the biological investigations advanced the chemical ones lagged behind This is undoubtedly due to the extremely complex nature of the material It could be freed from phosphorus and nitrogen and an oil containing only carbon, hydrogen and oxygen could be prepared Its actual chemical nature could not be determined since the substance even in its purest form obviously was still composed mainly of contaminants

Every attempt to crystallize the material at this stage met with failure The placenta was found to be a good source of the material, and estrus producing hormone prepared from placenta appeared to have the same properties as that prepared from ovaries but it still resisted all attempts at purification It was not until Aschheim and Zondek (2) in 1927 showed that pregnant animals of certain species excrete large quantities of the estrus producing hormone that real advances were made This astonishing discovery can truly be said to have changed the whole face of research in the field of endocrinology as applied to sexual physiology The urine of many pregnant mammals contained two hitherto unsuspected hormones first one capable of stimulating the ovarian follicle—the presence of which forms the basis of the well known Aschheim and Zondek reaction—and second the estrus producing one The urine of a pregnant mare was found to be a particularly good source and commercial interests in Germany arranged for collection of this material together with its concentration and extraction with a volatile solvent The oil that resulted was found to be powerfully estrogenic and not contaminated with the fatty substances that made the original ovarian material of Allen and Doisy impossible to deal with chemically Workers all over the world therefore turned to this material

II THE STUDY OF THE RELATION OF CHEMICAL STRUCTURE TO ESTROGENIC ACTIVITY

Historical background of estrus producing substances

Before it is possible to describe the work leading to the discovery of the potent synthetic estrogens we know today, it is necessary to give a historical background of the whole question of estrus producing substances The cyclical changes associated with reproduction in female animals are dependent primarily upon the ovaries The earliest agricultural experimentalists knew that any interference with ovarian function meant almost certain loss of reproductive ability With the development of physiology and histology in the eighteenth and nineteenth centuries, it became obvious that the ovaries played a more subtle part in reproduction than the mere producing of eggs, although of course this is the essential part of the whole phenomenon No real progress could be made until a clear concept of the science of endocrinology was formed, and we can therefore say that no real advance in a full understanding of the cyclical processes of the female were possible until this century Precise knowledge of the cause of estrus dates from the classical investigations of Allen and Doisy in the early 1920 s (1) This combination of a biologist and a biochemist was capable of solving problems and laying foundations which neither alone could have done Throughout these lectures we shall call attention to a number of other advantageous combinations of persons of different disciplines Edgar Allen was able to bring to the problem a detailed knowledge of the estrus phenomena of laboratory rodents as studied by the smear method of Papanicolaou (13), while Doisy was able to contribute that expert knowledge of biochemistry that has made him one of the world s leaders in this subject

Very briefly you will be reminded that they were able to re

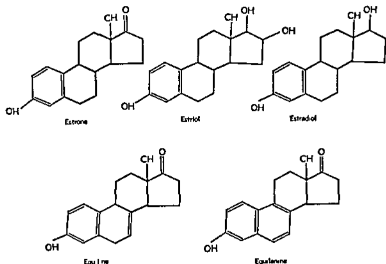


Figure 9

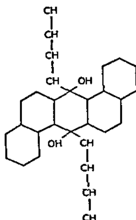
cal activity have been found in a body secretion For a time it had been assumed that the organs of internal secretion used only one hormone for their specific controlling function—the pancreas produced insulin the suprarenal medula produced adrenalin the thyroid produced thyroxin—and the idea that a number of hormones with the same biological activity yet differing in constitution could be produced was an entirely novel conception Today we know that this is by no means limited to the internal secretions of the sex glands Thus a whole series of new substances secreted by the thyroid have been discovered by Mrs Pitt Rivers (12) by Bruce Pitt Rivers and Sloviter (3) and by Thibault and Pitt Rivers (14), and the importance of nor adrenalin is becoming increasingly recognized It is also interesting to speculate on the significance of the work now being performed in Professor Marrian s laboratory in Edinburgh which has shown (11 15) that there are other unsuspected estrogenic substances present in normal and pathological urine This is all the more astonishing when one considers the enormous amount of careful analytical work that has been done on the urine of mammals since the early 1930 s It was the multiplicity of estrogenic substances in the urine of

By 1930 a number of workers had succeeded in crystallizing this substance first obtained by Aschheim and Zondek Naturally, as soon as the substance had been obtained in a pure form, intensive research on its constitution commenced It very soon became apparent that there were discrepancies among the different groups of original observers These were at first thought to be due to error, but careful comparison of crystalline material and analytical data soon convinced the workers in this field that there were certainly more than one and possibly several substances, in the urine of the pregnant mare all capable of producing estrus, and differing in constitution Fortunately, at this period another group of organic chemists had definitely established the basic character of the formula of the sterols, and cholesterol was at last assigned a definite constitution The basic part of the cholesterol molecule consists of the well known cyclopentenophenanthrene ring system, and it was shown that the sterols differed from each other in the distribution of double bonds hydroxyl groups, methyl groups etc, attached to this basic skeleton It is quite certain that without this knowledge the constitution of the sex hormones might never have been discovered and in any case would have been indefinitely delayed

A similarity between the crystalline estrogenic substances and the sterols was noticed by all workers and it was suggested that they might have something in common By the early 1930's the constitution of the estrogen present in the greatest quantity in the urine of the pregnant mare was definitely decided upon This substance, known as estrone, was characterized by the same basic cyclopentenophenanthrene ring system as that in the sterols In all, four substances were found in the urine of the pregnant mare and one in the follicular fluid (estradiol) These are shown in Figure 9, which demonstrates the structural relationship existing between estrone, estradiol estriol, equiline and equilenine Estradiol has not been found in the urine of pregnancy but was later found in the follicular fluid It was interesting however, that it was first prepared in the laboratory by the catalytic reduction of the ketone group in the 17 position in estrone We thus have the production of a naturally occurring substance by the laboratory before it is detected in nature The significant point is that for the first time a whole series of substances with the same basic biologi

by reference to the feathers from similar parts of the body of a hen Careful histological examination showed that in the case of both mammals and birds the effect of 1 keto 1 2 3 4 tetrahydro phenanthrene was exactly the same as that of estrone or estradiol from the purely qualitative point of view Of course the quantitative relationship was a very different story In the case of estradiol it requires roughly 1–1 5 μg to produce a full estrus response in the types of rats that we were using The relatively poor activity of our compound can be gathered when one considers that it required 100 mg to do what could be done by 1 μg of the naturally occurring substance To many this seemed to relegate our observation to one of pure academic and minor importance but to us it meant something much more namely that this very complicated biological lock could be picked by a strange type of skeleton key While the original key might be a delicate and flimsy affair it had to be admitted that our skeleton key was a very cumbersome one but the fact that the lock could be picked and not forced was an indication that we might be dealing with a new biological phenomenon

A very large number of phenanthrene compounds were investigated by us The most active compounds were obtained in a series of substituted dibenzanthracene diols The general formula of these compounds is shown in Figure 11 and it can be seen that



pregnancy that led Professor J W Cook and myself to consider how far one could depart from the molecule of estrone and still retain estrogenic activity We argued on the fact that, since nature produced five substances of differing constitution, all with the same biological activity, from the qualitative point of view though differing quantitatively, it might be possible to produce substances synthetically of a widely differing constitution We decided to experiment with oophorectomy rats to see the effect on the naturally occurring hormones of injecting substances of roughly similar constitution We first investigated the possible activity of phenanthrene compounds since this nucleus is common to all the naturally occurring estrogenic compounds We fixed our dose of compound arbitrarily at 100 mg This figure was chosen as it was shown that this could be conveniently dissolved in oil and could be administered to rats over a period without producing ill effects The first evidence of activity was found in the compound 1 keto 1 2 3 4 tetrahydrophenanthrene (Fig 10) This substance

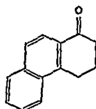


Figure 10 —1 keto-1 2 3 4 tet ahyd ophenanthrene

though possessing the phenanthrene nucleus and 1 keto group differs vastly from the naturally occurring molecule of estrone An amount of 100 mg of this material produces a full estrus response in ovariectomized rats and later on it was shown to possess all the properties of estrone itself These results were published in 1933 (5) A very striking demonstration of the activity of this substance was shown in the effect on the feathers of the brown leghorn capon In the capon the feathers are of the ordinary male type, but if an estrogen is injected, characteristic alteration occurs in the color of the feathers The injection of 1 keto 1 2 3 4 tetrahydrophenanthrene into a brown leghorn capon produces the characteristic feminization of the feathers which is demonstrated

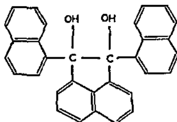


Figure 12 — 1,2 dihydroxy 1 2-di α naphthyl acenaphthene

We decided to investigate the activity of substances of a simpler constitution, and we therefore turned to α-naphthyl carbinol This was also found to be active at about the same level as the acenaphthene derivative Attempts to simplify still further the constitution by studying the activity of triphenyl carbinol were without success We thought that we would consider the possibility of activity in relatively simple substituted methanes and ethanes We were very pleased indeed to find activity in compounds such as diphenylethane and a higher degree of activity in 4 4 dihydroxydiphenylethane The activity of these compounds was usually greater than that of the original 1 keto 1 2 3 4 tetrahydrophenanthrene and lay between 100 mg and 20 mg per rat dose as tested by our method We decided to investigate the effect of unsaturating the compound and a series of investigations were performed studying derivatives of ethylene The first compound tested was stilbene or diphenylethylene This was found to possess definite though rather feeble activity As we had shown in earlier experiments that the hydroxylation of the phenyl groups resulted in an increased activity we prepared 4 4 dihydroxylstilbene and found this to be very much more active than the parent compound If one of the hydroxyl groups is dropped off then the compound still retains its activity although it is not so potent as the dihydroxy derivative We can now reveal the position that we had arrived at in the relation of structure to estrogenic activity at this period At first experiments and conditions had shown that the phenanthrene ring system was not necessary and that activity could be obtained when one had present two benzene rings eparated by an aliphatic block Maximum activity was found at

they are dialkyl diols of dibenzanthracene If the length of the alkyl side chain is altered very differing biological properties appear Thus the maximum activity occurs when the dialkyl substituent is the dinormal propyl Activity decreases if the length of the side chain is increased beyond this or diminished, and we have here an orderly series of compounds with a smooth range of activity

So potent was the dinormal propyl substituent that its activity was roughly the same as that of the naturally occurring substance *estriol* *Another interesting observation was that the dialkyl sub* stituent was completely inactive When we discuss the highly active compounds that were discovered later, we will see that minor alterations in the molecule produce very great changes in activity in exactly the same way as in this series

As we look back it is rather strange that these observations did not attract more attention at the time The only explanation is that these compounds are extremely difficult to make and it would, therefore be a considerable undertaking for any organic chemist to confirm the results One can only assume that they were regarded, perhaps justifiably with some suspicion until they could be repeated Up to this time all the experiments had been conducted in collaboration with J W Cook (4 5 6) The pressure of other duties made it impossible for him to continue and the work from this period was conducted solely in the Courtauld Institute of Biochemistry in the Middlesex Hospital Medical School

Together with my colleague W Lawson I decided to investigate the possibility of activity in compounds which did not possess the phenanthrene nucleus It was naturally difficult to know which type of compound to choose as a start for this investigation After careful speculation we decided to investigate the possibility of activity in derivatives of acenaphthene The basic skeleton of these compounds contains three rings but arranged in a different manner to phenanthrene We were very pleased indeed to find a considerable degree of activity in a di α-napthyl derivative in effect 1 2 dihydroxy 1 2 di α napthyl acenaphthene This compound is shown in Figure 12 It possessed a higher degree of activity than the 1 keto 1 2 3 4 tetrahydrophenanthrene and again was shown to possess, qualitatively all the activities of the naturally occurring substance

trast with many of the compounds whose activity we had pub lished the preparation of anol was very easy Practically any per son with the ordinary facilities of a chemical laboratory could pre pare an adequate quantity of material for testing and this a large number of workers did We very rapidly began to receive infor mation that our paper had met with a mixed reception A few workers confirmed our findings but the majority of them reported either no activity in the specimens they had prepared or very little We quickly realized that something very serious was wrong and we prepared a whole series of separate batches of anol from dif ferent batches of anithole and subjected them to a thorough bio logical investigation We were dismayed to find that our critics were correct in that it was possible to obtain samples of anol which possessed either no activity at all or very slight, up to high activ ity We then commenced a period of most anxious investigation in the laboratory to try to correct this serious error It was obvious that activity was present in some specimens of anol and we had in fact supplied some for clinical trial and had obtained the most encouraging reports from a very skeptical clinician who used the material in the treatment of menopausal symptoms We found ourselves in perhaps the most maddening position of a research worker knowing with certainty that activity is present but that it is not due to what we originally claimed Theoretical considera tion of the position would indicate that the possible explanation lay along the lines of a contaminant produced by polymerization of the parent substance It is well known that the configuration of anol possessing as it does a double bond in the side chain and a hydroxyl group in the para position is particularly conducive to polymerization If anol is left at room temperature or in the pres ence of air for any length of time it will change from a white crystalline substance into a dark oily material this undoubtedly being due to the formation of polymers We felt that the poly merization might have occurred during the process of demethyla tion and that the mother liquor from which the phenol was finally separated might easily contain this polymer An investigation of the mother liquor showed that it contained a substance soluble in organic solvents of a glassy character but possessing very high estrogenic activity The estrogenic activity of this material was

the ethane series and any increase or decrease in the length of the aliphatic chain between the two nuclei resulted in decrease or loss of activity Finally the activity was greatly increased by the introduction of a hydroxyl group in the benzene nuclei in the para position Introduction of a second hydroxyl group in any of the other positions resulted in a definite loss of activity, while any other position than the 4 4- or para resulted in a considerable diminution of activity The most potent compound, 4 4-dihydroxystilbene, still only possessed a fraction of the activity of the naturally occurring substance, and therefore it is quite obvious that there was no possible practical use of any of these compounds

Encouraged by the success of our activities in simplifying the molecule, we decided to go one stage further and try to eliminate one of the benzene rings in 4 4-dihydroxystilbene It was proposed to replace this with a methyl group, and the compound that we required was therefore parahydroxypropenylbenzene Now this substance could be readily obtained by heating anethole in a sealed tube with an alcoholic solution of potassium hydroxide This produces demethylation, and the phenol parahydroxypropenyl benzene can be easily separated in the classical method This substance is well known under the name of anol (Fig 13) We

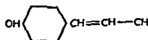


Figure 13 —Anol

prepared it by the method stated above and examined its activity in oophorectomized rats the compound appeared to be highly active A series of experiments at this time showed us that the compound possessed activity approaching that of the naturally occurring hormone This seemed a very successful conclusion to our attempt to synthesize the molecule and to finish up with one so readily available appeared to be an ideal solution to what looked originally like a very complicated problem

When we published these results (7), we had little idea that we were letting ourselves in for an extremely complex investigation that was not without its moments of extreme anxiety In con

Synthesis of stilbestrol

We must now turn to the activities of another group of workers quite independent of the Courtauld Institute of Biochemistry Sir Robert Robinson had been interested for some considerable time in attempts at a total synthesis of a steroidal molecule His object was eventually to produce a synthesis of the estrone molecule and from there to proceed to the synthesis of other allied substances During this period he had studied ring closure on various models and among them he had prepared the dimethyl ether of the symmetrical dimeride of anol This is shown in the formula in Figure 15 and it can be seen that this would be a very suitable

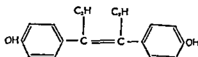


Figure 15.—Stilbestrol

substance to investigate from the point of view of ring closure to form chrysene After discussion with Sir Robert Robinson it was decided that the group of workers under him in the Dyson Perrins Laboratory at Oxford and those of the Courtauld Institute should immediately join forces in an attempt to be the first to synthesize the symmetrical dimeride which we had every reason to hope might be the wanted substance We demethylated the compound in the same manner as that used for the production of anol by heating in a sealed tube with potassium hydroxide and alcohol and then separating the phenol in the usual manner The demethylation went very smoothly and a white crystalline solid with sharp melting point was obtained When the activity of this compound was investigated it became apparent that we had obtained a substance of astonishing potency Whereas 100 mg of our first synthetic estrogen was required to produce the changes in a rat less than a fifth of a μg appeared to be active in the case of this substance It was roughly three times more potent than the naturally occurring hormone From the purely theoretical point of view even this substance was not sufficiently potent to explain away our original results since too great a concentration of this symmetri

certainly in excess of 0 1 mg as a rat dose as judged by our methods This substance was consequently isolated from the mother liquors of all batches of anol, and we assumed that it contained the polymer which was contaminating some of the batches of anol found to be active Out of this knowledge we immediately published a retraction of our claim for the activity of anol and pointed out that our mistake was undoubtedly due to the adsorption in small quantities of a substance capable of conferring activity on an otherwise inactive substance If this assumption were to be correct, then the actual contaminant would have to possess a fantastic activity as judged by standards at that period It would have to be more active than the naturally occurring substance since we had found activity in the very small quantities present in the specimens of anol that were active Our position was far from enviable since we had demonstrated by our error that it was possible to produce a substance of very high activity by the relatively simple process of demethylating anethole We had also had to point out that this was probably a polymer of anol and might very easily be a dimeride Since the production of such a substance could have very great clinical and, therefore commercial possibilities, it was obvious that all those who had interest in this group of therapeutic agents would commence work to try to produce the active substance and then protect it with patents The launching of such a substance in the ordinary course of publication would obviously have very severe repercussions on the whole industry concerned with the production of estrogenic therapeutic agents One dimeride of anol was known at the time and was the asymmetrical one known as dianol (Fig 14) The investigation of this proved that it could not be the compound we required since its activity was not approaching that of the naturally occurring hormone

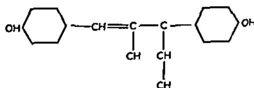


Figure 14 —Dianol

III BIOLOGICAL ACTIVITY OF THE ESTROGENS

As described in the second lecture the substance known as stilbestrol was synthesized by a relatively simple process starting from anisaldehyde The production of stilbestrol has been very widely studied by organic chemists throughout the world and there are many alternative methods of synthesis to the one described by us

Potent as this substance is, however it is not sufficiently so to explain the activity of our early samples of anol Something more potent would have to be discovered if our concept of con tamination was to be supported We, therefore, turned to a study of the mother liquor from the demethylation of anethole By remethylating the material we were able finally to separate out another substance which proved to have very similar properties to stilbestrol Subsequent work (6–8) showed that this compound was in effect a hydrogenated form of stilbestrol and is known as hexestrol (Fig 16) It is in effect stilbestrol with the ethylene

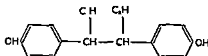


Figure 16 —Hexestrol

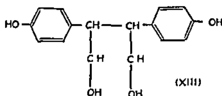
linkage converted to ethane by the addition of two hydrogen atoms As we shall see later this produces the possibility of stereo-isomerism and we now know that the highly active form of hex estrol is the meso compound This substance when tested on rats was found to be more potent than stilbestrol and to have a degree of activity sufficient to account for our early fallacious results We therefore feel convinced now that the high activity of some of our specimens of anol was due to their contamination with hexestrol

cal dimeride would be required for the contamination of the anol in order to give it the potency that certainly occurred in some of our early preparations The synthesis of the symmetrical dimeride was described in publications in 1938 (8–10), and we proposed the name diethylstilbestrol for the compound We regarded 4 4-dihydroxystilbene as the parent substance, which we proposed to call stilbestrol We felt at the time that it might be possible by varying the lengths of the side chains of the α α -carbon atoms to obtain substances of even greater potency than the diethyl substituent As we shall see later, this was not to be, and the substance was passed into common usage under the name of "stilbestrol" and we shall refer to it as this in future

The synthesis is described as starting from anisaldehyde and is a relatively simple one to perform All the starting material is easily obtainable and inexpensive

possesses 1/100 of the activity of the free phenol and the mono methyl ether is also considerably less active The introduction of any other substituent in the benzene rings is followed by an imme diate loss or decrease of activity, while the removal of the hydroxyl group from the 4 4′ position to any other in the ring is again attended by a very significant drop in activity The hydrocarbon itself possesses less than 1/1000 of the activity of the hydroxylated compound

It is interesting to note that while the experiments described up to the present have failed to increase the activity, a number of other experiments in which minor alterations were made to the molecule resulted in total loss of activity Thus ring closing through the two alkyl groups producing a cyclohexane ring causes complete loss of activity Another very interesting observation was made by showing that if one introduces hydroxyl groups into the terminal carbon atoms of the side chain the resulting compound is again completely inactive This substance 3 4 di p(hydroxyphenyl) 1 6 dihydroxy hexane, is shown in Figure 18



Figu e 18 — 3 4 di p(hyd oxyphenyl) 1 6 d hydroxy he a e

and has been the subject of a great deal of investigation Even when given in doses of 100 mg it proves to be inactive Its phar macological activity was very thoroughly investigated and it was shown to have no action whatsoever on the anterior lobe of the pituitary and none at all on experimentally raised blood choles terol

The question of optical activity has also been studied Hex estrol, containing two asymmetric carbon atoms can exist in sev eral forms the meso, or internally compensated form which is the most active and which has a melting point of 185° C and an activity of 0 2 gamma and the racemic form which has a melt

produced during the demethylation of the anethole These experiments were very quickly repeated throughout the world and our results were immediately confirmed by other workers Despite the fact that stilbestrol was announced in the middle of 1938 (see my second lecture) when the clouds of war were already gathering, it is interesting to note that it was introduced almost immediately into clinical practice Its use has grown from that date until at the present time it has a very wide range of usage both in the veterinary and in the human field This we shall discuss at a later date

There now commenced in my laboratories a period of intensive preoccupation with this series of compounds Chemical work was carried on in collaboration with Sir Robert Robinson and his colleagues, and intensive biological investigations were conducted in the Institute From the chemical point of view a very large number of modifications of stilbestrol were made Experiments were performed in which the side chain on the α α'-carbon atoms was modified in hope of further increasing the activity, and the results showed that any departure from the diethyl substituents resulted in either a loss of activity or complete disappearance It is interesting to note that the introduction of double bonds into *the side chain is not attended by any loss of activity* For example, the hexadiene shown in Figure 17 is highly potent and has been

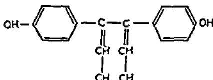


Figure 17 —Dienestrol

extensively used clinically It was originally called by us "Dienestrol" and as such it is known in the clinical field Introducing a double bond at the end of the chain, in other words, the divinyl substituent produces no change in activity the resulting compound being as active as stilbestrol itself The replacement of the ethyl chains by cyclopentyl rings is attended with loss of activity Any interference with the position of the hydroxyl group is also attended by a severe drop in activity The dimethyl ether only

TABLE I*

SUBSTANCES IN WHICH THE DISTANCE *D* IS VERY NEARLY THE OPTIMUM 8 55 A THESE SUBSTANCES SHOW THE HIGHEST ESTROGENIC ACTIVITY

Substance	D stance (A.)	Acti ity (R U)
Trans-4 4 dihydroxy α β diethylstilbene	8 55	3 γ
Trans 1 2 di α (4-hydroxynaphthyl) 1 2 diethyl ethylene	8 56	<10 γ
1 methyl 2 (4 hydroxy phenyl) 3 4 dihydro-6 hydroxynaphthalene	8 56	5 γ
3 9 dihydroxy 5 6 11 12 tetrahydrochrysene	8 75	10 γ

After Schu ler (33)

TABLE II*

SUBSTANCES IN WHICH THE DISTANCE *D* IS APPRECIABLY LARGER THAN 8 55 A THESE SUBSTANCES SHOW DECREASING ESTROGENIC ACTIVITY IN PROPORTION TO THE DEVIATION FROM THE OPTIMUM DISTANCE 8 55 A

S b ta ce	Di tan (A)	Act v ty (R U)
1 3 di (4-hydroxyphenyl) 1 2 diethylpropane	9 8	5 mg
1 4 di (4 hydroxyphenyl) 2 3 diethylbutane	12 0	Inactive
2 8 dihydroxy 5 6 11 12 tetrahydrochrysene	9 45	150 γ

After Schu l (33)

TABLE III*

SUBSTANCES IN WHICH THE DISTANCE *D* IS APPRECIABLY LESS THAN 8 55 A THESE SUBSTANCES ALSO SHOW DECREASING ESTRO GENIC ACTIVITY IN PROPORTION TO THE DEVIATION FROM THE OPTIMUM DISTANCE 8 55 A

S bstance	D t nce (A)	Acti ity (R U)
Trans 3 3 dihydroxy α β diethylstilbene	7 7	Less than 4 4 analogue
Trans 2 2-dihydroxy α β diethylstilbene	5 9	Less than 3 3 analogue
p p dihydroxydiphenyl-ether	8 0	100% estrus with 80 mg
p p dihydroxyphenyl	7 1	100% estrus with 100 mg

Aft Schu ler (33)

ing point of 128° C and a much lower activity of 100 gamma The dextro variety has an activity of roughly 100 gamma, while the levo is much less active, being under one tenth that of the dextro variety In the case of stilbestrol it is possible to have two varieties the trans which is the most active form, and the cis, whose activity is only about one twentieth that of the trans

In the case of the stilbestrol hexestrol series it is tempting to speculate on the similarity of structure of stilbestrol to the naturally occurring estrogens If written as in Figure 19, the appear

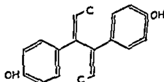


Figure 19

ance is suggestive but work in 1948 by Jeffrey, Koch, and Nyburg (24) would suggest an entirely different orientation as shown in Figure 20 An interesting suggestion was made by

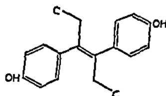


Figure 20

F W Schueler in a publication in 1946 (33) where an attempt was made to correlate certain molecular dimensions with estrogenic activity Schueler points out that in 1941 Giacomello and Bianchi (14) showed that the distance between the hydroxyl groups in stilbestrol and between the ketone and hydroxyl group in estrone were the same namely 8 55 A This he described as the optimum distance and designated it by the letter *D* On the basis of this he separated estrogenic substances into four categories (Tables I–IV) An inspection of these tables would certainly

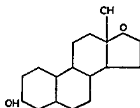


Figure 21 —Sterol breakdown

this consideration which was later developed with extremely in teresting results by Meischer and his collaborators (29) They were able to produce highly active substances in which the five membered ring had been opened These are shown in Figure 22

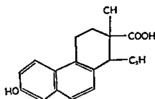


Figure 22 —B sdehydrodo synol c ac d

The compound was named bisdehydroisynolic acid by Meischer This substance presents a very high degree of activity but when it is under the same conditions as stilbestrol it is found to be less active

Again pursuing this interesting degradational hypothesis Ho reau and Jacques (22) went one stage further and produced com pounds in which in addition to ring D being opened ring C was also disrupted These naphthalene derivatives were named alle

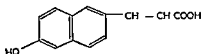


Figure 23 —All nol c ac d

TABLE IV*

SUBSTANCES WHICH HAVE THE PROPER DISTANCE *D* BUT POSSESS NO HYDROXYL OR KETO GROUPS

Substance	Distance (A.)	Activity (R.U.)
Triphenylchloroethylene	8 56	65 γ

* After Schueler (33)

indicate considerable support for Schueler s ingenious suggestion but a review of other estrogenic substances already listed, would indicate that his theory cannot by any means explain the relation ship between structure and activity For example, experiments with lengths of the side chains attached to the α α -carbon atoms clearly show that these can, so to speak, make or break the estrogenic activity despite the fact that *D* remains the same in each case *Another potential argument against this theory is the high activ ity* of certain of the dibenzanthracene diols described earlier Here the distance between the two hydroxyl groups must be less than 4 A It would appear, therefore, that while Schueler's hypothesis might apply to certain types of estrogenic compounds it cannot be applied as a theory to account for the whole of the activity

An interesting review of this subject will be found in a *Sym posium of Steroid Hormones*, edited by E S Gordon in 1950 (20) At the present time we have no explanation for this astonishing variety of compounds and it is obvious that much more research must be done in this field if we are to establish the real reason why organic compounds possess estrogenic activity Until this is done we shall certainly have no proper understanding of estrogenic activity nor is it likely that we shall ever understand the mechanism of carcinogenesis

While the stilbestrol series of synthetic estrogenic agents has had the most attention from biological and clinical workers there have been a number of other substances produced with estrogenic activity When it was shown (10) that 4 4′ dihydroxy diphenyl possessed definite activity it occurred to us that some of the syn thetic compounds might represent breakdown products of the cyclopentenophenanthrene ring system It was envisaged that this might occur as shown in Figure 21 The discovery of the highly active, substituted stilbene derivatives turned our attention from

This of course is an entirely new comer to the range of estrogens The formula shown in Figure 24 is very obviously related

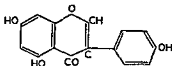


Figure 24—Genistein

to stilbestrol in fact, if the oxygen bridge is removed the formula of stilbestrol is presented Lawson has conducted a series of researches in which he has modified the structure of the naturally occurring compound and has produced a highly active substance (27)

In a review of this type it is impossible to mention every published compound which has been shown to have estrogenic activity but those mentioned above indicate the series of compounds in which high estrogenic activity has been found to exist

To Lacassagne (25) belongs the credit of being the first to use a labeled atom in a synthetic estrogen We have already referred to the halogen derivatives of triphenylethylene and by introducing isotopic halogens into this compound Lacassagne was able to study the effect of a labeled estrogen A considerable amount of work has been done by labeling in the stilbestrol series The biological results have been disappointing as practically the whole of the material is excreted unchanged and there is no clear cut evidence of a breakdown or metabolism of the molecule A full review of the substances with estrogenic activity has been published by Solmssen (34)

Biological activity of the estrogens

Almost without exception the estrogens are estimated by the laboratory process originally introduced by Allen and Doisy over thirty years ago (1) This consists essentially of studying the amount of material to be injected which is capable of causing cornification of the vaginal epithelium in the castrated laboratory rodent The height of estrus is demonstrated by the well known smear method of Papanicolaou (35) Despite the fact that oopho

nolic acid and are shown in Figure 23 Though possessing a high degree of activity, they are not so active as the stilbestrol series

After the activity of stilbene had been demonstrated (10), Robson and Schonberg (32) showed that triphenylethylene possessed definite, though much weaker estrogenic activity This compound had the novel feature of producing a very prolonged estrus, possibly due to its absorption into the fat depots of the body Later they were able to demonstrate an increased activity with halogen substituted derivatives Thus triphenylchlorethylene is said by Robson and his colleagues to be twenty times more active than the corresponding triphenylethylene Lawson (26) has recently returned to a study of the activity of simple phenolic substances described originally by Dodds and Lawson (12) He was able to show undoubted but weak activity in para tertiary amyl phenol The greatest care was taken to be certain of the purity of this compound and Lawson's work shows very definitely that the activity is due to this particular configuration This then must be regarded as the simplest substance capable of producing estrus changes

To complete the above review of the types of substance capable of estrogenic activity, we must add an entirely new series discovered by what must be regarded as a novel approach to a chemical discovery It had been known that certain pastures in Australia reduced the incidence of fertility of sheep A careful examination by Bennetts and Underwood (4) showed that the cause of the infertility lay in the ewes, and an examination of the uterus showed an endometrial hyperplasia similar to that occurring in endometriosis This condition may be associated clinically with the administration or production of excessive estrogens in the body and Bennetts and his colleagues therefore predicated that some constituent of the pasture contained a powerful estrogen It was noted that these pastures were rich in subterranean clover and it was shown that an extract of this plant contained a highly potent estrogen Extensive work was commenced to isolate and identify the substance, and this was eventually completed by a brilliant series of researches in Perth by Bradbury and White (5) They isolated genistein (5 7 4 trihydroxy iso flavone) This compound when fed by mouth produces the characteristic changes in the uterine mucosa which are responsible for the infertility

stilbestrol appears to be insoluble in water having about the same solubility as barium sulphate, some 4–5 mg per liter This saturated aqueous solution of stilbestrol would pass for distilled water and was acceptable to the animals This had sufficient activity to produce complete cessation of growth (30) It can be shown that this is not due to toxicity, loss of appetite, etc as the animals will respond immediately to injections of growth hormone

5 *Effect on mammary glands* —Estrogens have characteristic effects on the breasts of animals In the prepubertal animal administration will cause enlargement and development of the nipples Under certain circumstances lactation can be produced Large doses of estrogens when administered to the human lactating female will result in complete inhibition of secretion and this has found a very useful therapeutic employment of the drug to assist in weaning

6 *Effect on hormone-dependent cancer* —In the human clinical field administration of stilbestrol has a definite effect on certain forms of carcinoma (This will be considered separately)

7 *Effect on blood lipoids* (This will be considered separately)

8 *Effect on nutrition* —Stilbestrol appears to have very definite effects on animal nutrition as shown by increased utilization of food substances early maturity and an alteration in the distribution of fat and meat on the carcass (This will also be considered separately)

It will only be necessary to consider two of the above categories in detail—estrogens in carcinoma and estrogens in animal feeding—and to give a very brief outline of their effect in blood lipoids

Estrogens in carcinoma

The similarity between carcinogenic activity and some of the changes produced by the sex hormones had early been noted The intense epithelial proliferation caused by the administration of estrogens to an immature female rat produces canalization of the vagina in a very short time and the piling up of epithelial cells which then keratinize Histologically these changes are very similar to those occurring in the early stages of an epithelioma although of course there is no invasion beyond the basement mem

rectomized females could be brought into estrus, as judged by the vaginal smear method, they were still not acceptable to the male, and vaginal plugs indicating mating could not be obtained in these animals Marrian and Parkes (28) showed that many times the dose of estrogen required to produce a positive vaginal smear was necessary before the animals became capable of successful mating Biological work with the early extracts was very difficult indeed because being of an oily character they had to be injected dissolved in some oily medium, and this resulted in poor and prolonged absorption It was not until the crystalline, naturally occurring substances and the powerful synthetic estrogens appeared that it was possible to undertake accurate quantitative work The advent of stilbestrol and similar substances had the added stimulus of being highly active when given by mouth Extended study of these substances soon revealed that there were other biological activities apart from the vaginal effect The more important biological activities are shown as follows

1 *The induction of premature puberty in the laboratory rodent*—This is shown by the canalization of the vagina and the development of estrus in the immature animal

2 *Feminizing of the plumage of capons*—This has already been referred to in the case of the synthetic estrogens

3 *Development of the secondary sexual characteristics in other animals*—Shown by development of pubic and axillary hair, enlargement of the breasts and other characteristic female configurations

4 *Effect on anterior lobe of pituitary*—When given in large quantities estrogens have a characteristic effect on the anterior lobe of the pituitary This becomes enlarged as shown by an increase in weight and there is a characteristic alteration in the cytology of the pituitary From the functional point of view the estrogens appear to inhibit the secretion of a number of the anterior pituitary hormones Thus the gonadotropins and the growth hormone appear to be inhibited at source as shown by castration effects in both the male and female and by absence of growth in immature animals This latter effect was very beautifully demonstrated in my laboratory by R L Noble He replaced the ordinary drinking water of immature animals by a saturated aqueous solution of stilbestrol One can say that to all intents and purposes

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brane Quite early in our work on the synthetic estrogens, Cook and I (9) were able to show that a number of carcinogenic hydro carbons possessed feeble but definite estrogenic activity Thus, benzpyrene and a number of benzanthracene derivatives were shown to be in this category It must be admitted, however, that their estrogenic activity was very much lower than their carcino genic activity When tested by the standard method of painting the substance on the skin of mice over a long period, stilbestrol is found to have no carcinogenic activity No surface carcinoma has ever been produced by the application of stilbestrol in this way Lacassagne (25) was able to show that if large doses of estrone were injected into certain strains of mice from very early in their life, carcinoma of the breast developed in a large percent age of the animals and from this point of view we must consider the estrogens as possible carcinogens under certain conditions A great deal of investigation has followed upon this fundamental observation of Lacassagne and it has now been clearly proved that the estrogens will only increase the incidence of carcinoma of the breast in a strain of mice which has this propensity This is to say that if a strain of mice can be found which has no spontaneous incidence of mammary cancer, then estrogens will fail to produce it, on the other hand, if offspring from a strain of mice having a low incidence of carcinoma of the breast are treated then there will be a much higher incidence Precisely similar effects can be obtained by the administration of stilbestrol

The introduction of estrogens into the treatment of carcinoma of the prostate occurred through the brilliant work of Huggins in the early 1940's (23) This development is so well known that it will only be necessary to summarize it briefly From the days of John Hunter in the late eighteenth century, it was known that castration produced an alleviation in a small percentage of the cases of tumor of the prostate In Hunter s time of course there was no exact differentiation between benign and malignant en largement of the prostate When the histological differences of prostatic tumors had been worked out in the nineteenth century, it was realized that castration only benefited patients with malig nant enlargement of the prostate Up to 1940 the operation of castration for carcinoma of the prostate had varied in popularity That it was effective, at least in the early stages, there was com

plete consensus of opinion but the aesthetic objections to the operation were such that it never became popular nor did it become a standard method of treatment Huggins turned his experimental mind to the subject of prostatic diseases and using the acid serum phosphatase method developed by the Gutmans (21, 36) as a method of studying prostatic activity, he was able to show that in carcinoma of the prostate with active secondary deposits the acid serum phosphatase was high It was also shown in the dog that removal of the testes produced a drop in the acid serum phos phatase Huggins had the advantage of knowing the modern en docrine literature, and it occurred to him that all the beneficial effects of castration could be obtained by inhibiting the anterior lobe of the pituitary by estrogens Owing to the high activity of stilbestrol when given orally, Huggins thought it would be possible to obtain a more or less permanent inhibition of the anterior lobe by daily doses of stilbestrol It is now common knowledge that this form of treatment produces an immediate and dramatic effect in cases of carcinoma of the prostate The effect on a local lesion is almost instantaneous and a person with retention overflow getting up perhaps ten or twelve times a night is made completely comfortable in the space of forty eight hours The enlarged gland shrinks and the obstruction is removed Not only is this effect obvious on the primary lesion but the secondary lesions also bene fit This is shown by a shrinkage in size and by relief of pressure symptoms on the spinal column Huggins has always pointed out that this is in no sense a cure and the beneficial results are ob tained simply because the prostatic tumor is hormone-controlled That the beneficial effects of stilbestrol are due to inhibiting the gonadotropin secretion and thereby producing castration effect can be shown by the fact that injection of testosterone into such patients will cause an immediate return of symptoms Successful though this treatment is it is not without its disadvantages The immediate objections are impotence and extremely painful nipples in a number of cases The more serious objection is that after a time the growth appears to escape the controlling effects of the drug and proceeds on its destructive course This is shown by return of the local symptoms increased pain etc in the region of the secondaries and the presence of further metastases

While stilbestrol treatment is now regarded as initially 100

per cent successful in cases of carcinoma of the prostate, it can not be prophesied as to how long this beneficial effect will last Cases have come out of control within six months from the start of the treatment, while others have been successfully controlled for many years In the Middlesex Hospital we have had patients who have survived completely controlled for ten years, although it must be admitted that this is unusual A resistant patient cannot be controlled by more estrogens or by varying the type of estro gen, and we can now say definitely that the development to re sistance is probably due to a secretion of androgens by the cortex of the suprarenal On this basis Huggins suggested that uncon trolled patients should be subjected to bilateral adrenalectomy and that the subsequent development of adrenal insufficiency could be controlled by the administration of cortisone There now exists a large body of literature on this subject and it would appear that bilateral adrenalectomy, following castration and the use of estro gens, does result in the diminution of symptoms in a number of cases, but it is not universally successful and enthusiasm for this operation at least in our country, has certainly waned One can, therefore, conclude by saying that stilbestrol has a very definite place in the treatment of carcinoma of the prostate In many cases a few symptomless years can be obtained and this relief is indeed a great triumph It may be said that stilbestrol is the first substance ever to be administered by mouth having an effect on one form of cancer, apart from the highly toxic substances

It was only natural after the work of Huggins, that stilbestrol should be tried in a large number of other types of carcinoma, and from the early 1940 s on the literature is cluttered with de scriptions of the beneficial effects of stilbestrol on every type of carcinoma A very careful study of the clinical evidence shows that only one other condition apart from carcinoma of the prostate emerges in which any evidence of benefit can be obtained and this is in inoperable carcinoma of the breast The administration of stilbestrol to these cases produces beneficial results in some 20 per cent of the cases This is shown by an improvement of the local condition and by a diminution in the size of the secondaries The extent of this beneficial effect may vary from an apparent miraculous 'cure' where all the primary and secondary lesions disappear, to just a slight improvement as shown by decrease in

pain in the region of the secondaries and slight hindering of the growth of the primary lesion The former effect—complete dis appearance of the whole di ease—though rare, has been reported from a number of centers We ourselves at Middlesex Hospital have had a limited number of cases in which the whole condition has disappeared, only, unfortunately, to reappear at a later date A careful analysis has revealed that the beneficial effects are suffi ciently great to make stilbestrol treatment of inoperable carcinoma of the breast an essential part of the management of this tragic and we must admit hopeless condition at the present time It is very difficult to understand the action of stilbestrol in this condi tion particularly when we consider that carcinoma of the breast can be produced in animals by its administration in certain circum stances Some workers have feared the worsening of carcinoma but this has not materialized It has been suggested that testos terone should be given to the younger patients and stilbestrol to the older ones It would seem reasonable to suppose that the mate rial acts again by inhibiting the anterior lobe

Effect on blood lipoids

Recently the synthetic estrogens have been used in the treat ment of chronic arterial disease on the basis that this condition may be associated with alterations in the fat and lipoid metabolism It is only possible here to give a brief review of this theory as it does not concern our main thesis Many years ago Anitschkoff (2) showed that if rabbits are given daily doses of cholesterol with their feeding stuff they will rapidly develop profuse atheroma of the aorta and the appearance of the opened artery is very similar to advanced atheroma in the case of a human subject To the naked eye the intima is largely destroyed and replaced by glisten ing white plaques Histologically a deposit of cholesterol can be seen in the intima and the whole appearance is very similar to that of atheroma in the human subject An interesting point is that if the animals are left untreated this condition will entirely clear up Other workers have tried to extend this to carnivorous animals but have failed and this resulted in the work of Anitsch koff being regarded as of academic interest only During the last ten years further interest has been aroused in the distribution of lipoids in the blood in patients suffering from arterial degenera

tive conditions such as atherosclerosis and various forms of this disease, particularly those leading to coronary ischemia Thus Gofman (16–19) has studied the distribution of lipoproteins in the blood of patients with arterial disease and has claimed that he is able to demonstrate characteristic alterations in the lipoprotein components This alteration is often associated with an increased plasma cholesterol level *Extensive investigations have by and* large confirmed Gofman's work, and it is now recognized that in the majority of persons with arterial disease there is evidence of an alteration of the lipoids in the blood This has been associated with dietary habits and it has been suggested among other hypotheses, that excessive intake of cholesterol, as by the consuming of egg-containing foods, may be responsible It was only natural that attempts should be made to reduce this condition, particularly when it is encountered in people who as yet have shown no clinical evidence of arteriosclerosis It is a well known clinical fact that arterial degeneration in women is practically never seen during the active sexual life and that the condition is only common after the menopause From this it was concluded that perhaps the sex hormones have a definite effect in inhibiting those changes in the blood that may be responsible for the development of arterial disease Barr and his collaborators (3) were the first to suggest that the administration of estrogens might affect the blood lipoids There now exists a body of literature in which the effect of stilbestrol on arterial disease has been noted to the effect that the clinical *condition of persons with arterial diseases is improved by the ad*ministration of stilbestrol, but this of course is extremely difficult to prove It has also been claimed that administration of the drug over long periods reduces the tendency to coronary ischemia This again is extremely difficult to establish with any degree of certainty What has been shown however, is that the administration of stilbestrol will produce a complete change in the lipoprotein distribution in the blood This was found by Eilert (13), who showed that if a patient with characteristic alterations of lipoproteins is treated with stilbestrol a return to the normal distribution will occur in a relatively short time Oliver and Boyd (31) have studied the effect of estrogen administration on blood cholesterol and have shown that this produces a reduction A unit in my department is working on this problem and a full study of

the α and β lipoproteins and blood cholesterol is being undertaken by chemical ultracentrifugal and electrophoretic methods It is too early, as yet, to give any final results, but it can be stated that it is possible to change the pathological picture of the distribution of blood lipoids to the normal by the administration of stilbestrol

As in the case of carcinoma of the prostate adult active male patients complain of the inescapable side effects of stilbestrol therapy namely complete impotence enlargement of the breasts and particularly excessive soreness of the nipples Many writers have pointed out that if a substance can be obtained without these undesirable effects a great advance will have been made As we shall see later it is the view of the workers in my department that it is impossible to separate the estrogenic effects from the effect on the anterior lobe of the pituitary In other words, it would appear almost certain from our studies that the estrogen is producing the wanted effects namely action on blood cholesterol and lipoids on carcinoma of the prostate and on carcinoma of the breast

IV AGRICULTURAL APPLICATIONS OF SYNTHETIC ESTROGENS

That estrogens can have a powerful effect on cattle was clearly demonstrated by the work of Bennetts and his collaborators (5) on the subterranean clover, already referred to It will be remem bered that infertility of certain types of sheep was traced to ab sorption of an estrogen thought to be genistein due to the animals eating the subterranean clover It was only natural that when a highly active, cheap and orally employable estrogen became available it should be tried out on various forms of domestic ani mals and birds The first application of stilbestrol to food pro duction was its administration to old cock birds in the hope of making them salable as poultry Experiments were undertaken in which pellets of stilbestrol were implanted under the skin of the neck of these birds, and after some months it was noticed that they had increased in weight mainly as the result of the deposition of extra fat on the carcass It was also found that the birds were more acceptable as food since the flesh proved to be much more tender than that of the original old cock bird and resembled that of a capon Today this treatment is used extensively throughout the world and not only has been extended to old and unsalable members of the flock but has also been used for growing birds A single implant of 12–15 mg is used for a young bird while two pellets are used for the tougher ones The effects are noticeable within the first week and the best results are obtained after some four to six weeks treatment The method of administration has been largely empirical some using compressed tablets of pure stil bestrol and others tablets in which some filler base is used Very little work has been done on rate of absorption and optimum ab sorption It is thought that pos ibly about 2 mg per week are absorbed from a pellet of pure stilbestrol, but the effect of various

fillers and carriers in other tablets must have a profound effect on the rate of absorption

Tentative experiments have been made to try to find some form of master mix containing stilbestrol with a view to its being added to the poultry rations This has been the subject of inten sive investigations but up to the present no clear cut product has emerged from these researches Attempts have been made to use the same procedure on turkeys but the effects have not been so dramatic as in the case of fowls In the United States intensive investigation is being made into this interesting development and a full review appears in a recent report of the Committee of Ani mal Nutrition of the National Academy of Sciences

Anxiety has been expressed in some quarters that the consump tion of birds treated with estrogens might lead to a transference of the unwanted estrogen to the consumer In some countries the actual treatment with stilbestrol has been forbidden by law It is obvious that if the pellet is put well up in the neck there is little chance of the consumer being affected by the estrogen It must be remembered that the pellet always remains to some extent and therefore great care must be taken to throw away that portion of the neck containing the pellet

Synthetic estrogens in animal nutrition

The estrogen genistein is widely distributed in grasses but happens to be particularly plentiful in subterranean clover There fore an exclusive diet of this produced the symptoms of too much estrogen Estrogens have been recorded in grass and it has been suggested that the summer flush of milk may be due in part to the estrogens in clover It has also been known for some time that it is possible to extract from animal feeding stuffs substances of weak estrogenic activity The addition of estrogens to animal feeding as a direct experiment was first performed by Andrews and his colleagues (2) and published in 1949 They implanted pellets of stilbestrol of weight 12–24 mg in seventy pound lambs and studied the effect on rate of growth carcass quality and so forth There was a significant increase in the rate of growth but the carcass quality was definitely lowered These experiments were confirmed by a number of other workers who showed the rather unexpected fact that the stilbestrol treated lambs contained

less fat in relation to meat than the controls This of course is the opposite of what occurs in the chicken The most dramatic and striking results have been obtained in yearling beef heifers and steers Clegg and his collaborators (6) reported statistically significant increases in weight following the implantation of 60 mg tablets This work has been developed in the Iowa State College of Agriculture and workers there have developed a method of adding stilbestrol to cattle feed Roughly 10–20 mg per day is administered to the animals and it is reported that at this level there is an increase in the rate of growth of some 20–25 per cent and that the carcass quality is either unaltered or only slightly reduced It is said that these results are so striking that they can be seen and appreciated by the farmer without the necessity for statistical analyses I believe I am right in saying that today most farmers in the United States employ cattle feed to which stilbestrol has been added The results with pigs have not been so satisfactory and work is still in progress This form of administering estrogens is not without danger both to the animals and to the agricultural workers With the animals it is possible to obtain very undesirable effects If too much is given, softening of the pelvic ligaments occurs and frequently the development of nymphomania is a troublesome symptom A combination of these two frequently results in a fractured pelvis Another disadvantage of overdosage is a possible fall in milk production There is also very considerable danger to those engaged in preparing the material In the early days of the manufacture of stilbestrol in Great Britain adequate precautions were not taken to protect the workers and a number of cases of marked gynecomastia occurred in males working on the production of stilbestrol and particularly on the making of tablets A careful investigation showed that the main danger lay in inhalation of stilbestrol dust The substance itself is highly electric and flies about very easily The gynecomastia in the workers was very marked indeed and was accompanied by complete impotence These cases were studied very carefully and it was found that when they were removed from exposure the impotence disappeared and the breasts decreased in size but the enlargement never completely disappeared Today the greatest precautions are taken wherever stilbestrol is manufactured or packaged In the ordinary course of manufacture the penultimate stage

is the dimethyl ether This is relatively inactive and unless splashed about on the skin in large quantities it is unlikely to pro duce any untoward events After demethylation and crystalliza tion the substance becomes dangerous and precautions must be taken In most factories women are employed on this stage of the procedure and precautions of extractor fans airline masks etc , are taken This applies particularly to the making of tablets

It has been reported, unofficially, that gynecomastia has oc curred in some of the provender mills where stilbestrol is added to the animal fodder Adequate precautions can prevent this, but it would appear essential that only the finished feed and not the concentrated material should reach the farmer

As in the case of chickens the question immediately arises as to how safe is the consumer who eats the meat from treated animals On general principles one can say that, provided the estrogen treatment is left off a week before slaughter there is little likelihood of any significant quantities remaining in the body Extensive experiments with human beings have shown that three days after the cessation of administration it is very difficult in deed to detect stilbestrol in the excreta Experiments have been carried out to assess the extent of the hazard of eating meat from treated animals Such meat was fed to oophorectomized mice and the e trogenic effect studied by noting the increase in the weight of the uterus Mice fed with animal feed stuff to which stilbestrol had not been added showed a significant increase in weight of the uterus, thus confirming what was already known that the ration does contain weak estrogens By and large, the results on the meat feeding would tend to show that there was no hazard The best way to investigate this problem would be to feed animals on a diet in which labeled stilbestrol was substituted for ordinary stilbestrol The distribution of any remaining stilbestrol in the carcass could then be very quickly determined by the usual Geiger counter methods This experiment would settle the problem once and for all

The highly successful development of this theory of analogues of estrogenic substances has led many to speculate and to experi ment as to whether the reasoning and technique can be applied to other hormones Androgenic activity is known to be possessed by a very large number of compounds of the cyclopentenophenan

threne ring system, and on general principles it ought to be possible to construct an analogue for testosterone in the same way as a series has been made for estradiol Up to the present, however, no one has succeeded in producing a successful active compound with androgenic activity A number of claims have been made but careful investigation and repetition has shown that the results cannot be supported We ourselves have made a great many compounds with this end in view For example, we have hydrogenated, or partially hydrogenated, stilbestrol in the hope that the saturation and partial saturation, of the two benzene rings might result in a loss of estrogenic activity and a gain of androgenic activity While the estrogenic activity has been completely lost, we have never obtained any active androgens

In my laboratory we applied the analogue doctrine successfully to the carcinogenic hydrocarbons in one case If one considers benzpyrene and dimethylchrysene, both powerful carcinogenic hydrocarbons it is possible to imagine opening the rings to give a derivative of stilbene namely α ethyl β secondary butyl stilbene These three compounds are shown in Figure 25 Together

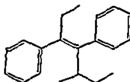


Fig 25 —α ethyl β secondary butyl stilbene

with Lawson and Williams (7) I prepared this compound and tested it out by the standard method of painting on the skin It was found that the stilbene derivative possessed definite but feeble carcinogenic activities A spindle cell sarcoma was produced in *some of the animals and* it was found possible to transplant this tumor into further generations of mice It is of course well known that the phenanthrene ring system is not essential for carcinogens, but it is interesting that this analogue does possess definite carcinogenic activity

Another partially successful venture on these lines was made in my department by myself in collaboration with my colleagues

Lawson and Williams (8) It will be remembered that the morphine series of alkaloids contains the phenanthrene ring system and we wondered whether it would be possible by imaginary openings of rings to produce compounds with morphine like activity Figure 26 shows the formula of morphine and by suitable opening

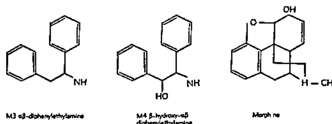


Figure 26

of rings it is possible to imagine diphenylethylamine as a possible analogue We made a series of derivatives and substituents of diphenylethylamine and tasted these for activity of a morphine like character We used the following criteria capacity to depress the righting reflex in rats raising the blood sugar level in rabbits and hyperexcitability pupil dilatation, and vomiting in cats A large number of these compounds were found to have these properties in varying degrees but the most highly active compound was β hydroxy αβ diphenylethylamine This was tested clinically in cases of pain due to secondary deposits of carcinoma and was found to possess a certain amount of activity in doses of 200–400 mg given every four hours It was subsequently shown that this material acted mainly on pain due to nerve pressure and did not possess clinically the global activity of morphine It is interesting however that some of the properties are possessed by these molecules

In conclusion it is difficult to say whether other biological actions can be imitated by analogues Up to the present little success has been obtained in this field other than with the estrogens and the plant hormones The naturally occurring hormone auxine can be imitated by a number of substances which have no structural resemblance to it Many of these have found their way to commerce as plant hormones for agricultural purposes In the

field of mammalian physiology and endocrinology, however, we must admit that apart from the estrogens the results have been disappointing This may be due to the fact that the other biological processes such as androgen activity and progestational activity call for greater specificity or that the investigators have not had the luck that attended those who worked on synthetic estrogens

Studies of the adrenal cortex

The isolation and characterization by my colleagues Mrs Simpson and Dr Tait (22), of a new adrenal principle, aldosterone (see fifth lecture), has been closely followed throughout the world and particularly in the United States Here physicians have been quick to seize upon this work and to use the properties of the new hormone to explain certain clinical syndromes and in effect to describe an entirely new series of diseases Before going into this work in detail, I shall sketch briefly the historical background of the suprarenal gland in relation to medicine and endocrinology in general These two small organs, situated at the poles of the kidneys, have always attracted attention because they appeared to have no obvious function They were originally called capsules by the old anatomists because at autopsy or dissection they were found to contain fluid due to the rapid putrefaction of their interior It was several centuries before anatomists decided that they were indeed ductless glands It was almost exactly 101 years ago that the first clinical interest in these organs was evinced Thomas Addison, the distinguished Guy s Hospital physician published a paper in the *Guy's Hospital Report* describing the autopsy findings on a number of cases all possessing the same clinical symptoms These symptoms consisted of pigmentation of the mouth and skin extreme lassitude and severe gastrointestinal upsets Post mortem examination revealed complete destruction of both suprarenal capsules and this was usually due to a tuberculous process These observations were quickly confirmed in the European institutes particularly in Austria and Germany where at this period post mortem anatomy had been developed in a very high degree The syndrome was almost immediately referred to as Addison's disease by people throughout the world, and by that name it is known today In the 1850 s there was no knowledge of internal secretion, and therefore an understanding of the mode

of action of the suprarenals was quite out of the question This observation of Addison s and its subsequent confirmation lay in the literature as a strange unaccountable fact From 1860 on similar strange observations began to appear For example the classical experiments of Von Mering and Minkowski published in 1889 (14), showed that removal of the pancreas in the dog was attended by the development of a condition indistinguishable from human *diabetes mellitus* The experiments of Kocher, the Swiss surgeon of Berne (13) showed that complete removal of the thyroid gland in animals and in man resulted in the develop ment of a disease which was indistinguishable from the well known clinical condition known as myxedema In the 1870 s Gull an other physician at Guy's (9) had described the association of myxedema and cretinism with absence or destruction of the thy roid gland By 1890 a general conception had developed that certain organs such as the thyroid and pancreas produced an effect on the body which could not be explained by any of the existing knowledge That there was perhaps something new and funda mental in this was emphasized by the classical experiments of Murray (15), who formulated the novel idea that, as myxedema occurred in persons without a thyroid they might respond to thy roid extracts when administered subcutaneously Murray per formed the first clear-cut clinical experiment in human endocri nology He produced a case of myxedema and showed it to a medi cal society so there could be no doubt about the diagnosis He then injected the patient with glycerine extracts of thyroid ob tained from the slaughterhouse The patient made a miraculous recovery and he demonstrated the same patient to the medical society again This was in the year 1891 and I have always felt that this pioneer did not receive the credit that was really due to him He of course was fortunate in that a very crude extract of thyroid was capable of producing these results Later he gave the material by mouth with the same results and again he was fortu nate in that the thyroid hormone is one of the few capable of resisting the processes of digestion and of being active by mouth The first animal experimental evidence of internal secretion was given by the classical experiments of Oliver and Schafer in 1895 (16) when they showed that an extract of the suprarenal gland was capable, when injected into an animal of causing an imme

diate rise in blood pressure Bayliss and Starling (4) actually used the word hormone after their discovery of secretin some seven years after the work of Oliver and Schafer By 1910 the concept of endocrinology was firmly established, and by 1924 one of the greatest triumphs, in the form of the isolation and production of insulin by Banting and Best (3), was to demonstrate the value of studies in this field It was only natural that attempts should be made to isolate from the suprarenal gland a substance capable of maintaining the patient in Addison's disease The internal secretion of the cortex of the suprarenal gland was studied, as it was now known that this was the part that produced the hormone whose absence caused Addison s disease, but these attempts lacked a suitable method of standardization In the case of insulin the investigators were lucky in having a relatively simple method of demonstrating the potency of their extracts They injected the extract into an animal and followed the blood sugar changes In the case of the suprarenal there is no such ready made and simple, direct method of testing Bilateral adrenalectomy is a difficult operation from which the operative mortality in most laboratory animals is high unless the worker is very skillful Again many animals have adrenal rests which are sufficiently active to maintain them even though the suprarenal glands themselves have been removed The rate of survival of adrenalectomized animals is extremely variable and it follows that any method of standardization based upon the length of time of survival must be attended with many dangers This led to various other types of tests such as those depending on exhaustion as for example the ability to swim for prolonged periods It is little wonder that no advance was made in this direction until the 1930 s Despite these great difficulties Swingle and his colleagues in Great Britain (18) succeeded in producing a stable and active substance from the suprarenal cortex obtained from the slaughterhouse This was standardized on adrenalectomized dogs and was of fairly uniform potency All the basic knowledge of the activity of suprarenal extracts is really centered on this classical work The constitution of Swingle s extract was not known and the type of process used would give a formidable mixture of compounds The extract was produced commercially and played a very important part in developing our knowledge of the clinical effects of suprarenal ex

tracts It is used successfully in the treatment of Addison s dis ease Its administration was able to control adrenal inadequacy Whereas the destruction of the capsules was almost invariably due to tuberculosis, this disease usually killed the patient despite the relief of the adrenal insufficiency

We must now turn to other workers those in the United States under Professor Kendall of the Mayo Clinic, and those in Basel Switzerland under the direction of Professor Reichstein These two groups working independently, succeeded in isolating from the suprarenal cortex a series of crystalline substances and in de termining their constitution These substances were shown to consist of a basic skeleton of the cyclopentenophenanthrene type exactly the same as that described in the case of the steroids and the estrogens The characteristic difference between the adreno cortical steroids and those of the sex glands is that, while the basic skeleton is the same, ring A is partially hydrogenated to a cyclo hexane ring and either a hydroxyl group or a ketone group is at tached to the 11 position The most important substances in this eries are corticosterone, dehydrocorticosterone and cortisone Their structure is shown in Figure 27 All of these substances are

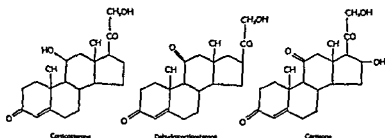


Figure 27

capable of maintaining the adrenalectomized animal alive and they possess all the properties of the Swingle extract It must be remembered that these substances were prepared in minute quan tities from relatively enormous amounts of slaughterhouse mate rial They remained academic curiosities of great interest to the laboratory worker but of no practical importance whatsoever We therefore have this interesting position of the clinical needs for

the treatment of adrenal insufficiency being adequately met by Swingle's extract of unknown constitution, yet obviously containing the substances whose structure had been worked out by Kendall and his collaborators (12) and Reichstein (17) This situation was quite satisfactory until the *work of Hench and his collaborators* (11) burst upon the world of medicine Rheumatism is too far removed from our field and theme to discuss the origin of Hench's work, but we know he was able to demonstrate that by the injection of one of these adrenocortical steroids cortisone it was possible to produce a remission in rheumatoid arthritis The great contribution of this work was to show that a pathological process, at one time thought to be irreversible, could be reversed in a relatively few hours by the administration of a hormone The result of the publication of the Mayo Clinic on cortisone was an *immediate world wide demand for this substance Its production* from natural sources was out of the question since the yield was almost of the order of milligrams from tons of slaughterhouse material We know the brilliant way in which this problem was solved by the pharmaceutical industry of this country While it is possible *to produce semisynthetically all the sex hormones by the* disintegration of cholesterol to dehydroisoandrosterone, and then to build up the required structure from this starting material, it is impossible to introduce a substituent into the 11 position of the cyclopentenophenanthrene ring system by any known chemical method today The question of total synthesis at that time was out of the question because, even if the structure could be arrived at synthetically, the question of stereoisomerism due to the large number of asymmetric carbon atoms presented formidable difficulties The problem was solved by starting with the bile acid deoxycholic *acid which in addition to other substituents, has a hydroxyl group* in the 12 position By a most elaborate chemical means it is possible to move this around, so to speak to the 11 position and then to trim the rest of the molecule to give cortisone This requires some forty steps of synthesis yet despite the complications cortisone was produced on a tonnage basis and is now a relatively common pharmaceutical product

When cortisone became available it was used extensively in the treatment of *rheumatoid arthritis and in those diseases known* as the collagen group Administration of cortisone was found to

produce, in addition to the beneficial effects in rheumatoid arthritis a number of side effects such as water retention and alteration of personality Hence a very careful study of the biological activity of cortisone and allied substances was necessary As the result of the intensive laboratory and clinical work it became apparent that the activity of the adrenocorticol steroid could be divided into two main categories as pointed out by the Canadian worker, Selye, some years before These activities were first of all referred to as 'glucocorticoid' and "mineralocorticoid ' The glucocorticoid activity refers to the effect of these hormones on carbohydrate metabolism They were found to favor the deposition of glycogen in the liver and to have a considerable effect on carbohydrate metabolism The mineralocorticoid action consists of a power conferred on the body to retain sodium and favors the excretion of potassium This property, if in excess, will lead to serious symptoms in the human subject, thus sodium retention can give rise to hydremic plethora which in turn can produce cardiac embarrassment and, in extreme cases edema of the lung and death Minor degrees of water retention can also cause impairment of the circulation increase in weight, and other alterations in metabolism Selye and his school maintained that it was possible to differentiate these two properties completely, but investigation of the existing corticoids up to the 1950 s indicated that they possessed both of these qualities in some one was more emphasized than the other Thus cortisone was mainly a glucocorticoid although it did possess some salt retaining properties Deoxycorticosterone, a semisynthetic product from cholesterol which is the same as corticosterone without the substituents in the 11 position was found to possess great salt retaining power and to be almost a pure mineralocorticoid This substance however up to 1956 not being found in nature, was in effect considered an artifact No pure mineralocorticoid had been found in the mammalian tissue and therefore many of Selye s theories which depended upon the existence of a powerful mineralocorticoid had to be regarded as hypotheses only

V THE DISCOVERY OF ALDOSTERONE

It was at this period that Mrs Simpson and Dr Tait became interested in this problem They conducted experiments on a preparation made on the lines of Swingle in Great Britain by Messrs Allen and Hanbury and known as "Eucortone" As this was prepared from whole glands, it therefore contained the "amorphous fraction"

Simpson and Tait had reviewed the methods for the estimation of mineralocorticoids and had realized that the existing techniques were quite incapable of being developed into a really quantitative type of method such as would be required if one were to work on the actual constitution of the substance After a number of experiments they devised an entirely new and unusual procedure in which they followed the alteration in the urinary ratio of sodium 24 to potassium 42 in the adrenalectomized rat By the use of this method, although not specific, they obtained a dose response curve which gave them extremely accurate determinations of mineralocorticoid activity By this technique they could detect minute traces of deoxycorticosterone when using this substance as a standard

Applying this method to the preparation 'Eucortone' they were surprised to find that it had a higher activity per unit of dry weight than any of the crystalline steroids with the exception of deoxycorticosterone A careful examination of the Eucortone for this substance failed to reveal its presence and it would appear that the results could be explained only by the presence of an entirely new and hitherto unsuspected mineralocorticoid or by a synergistic action of the mixture of known compounds Until the former could be isolated the latter hypothesis could not be excluded

Simpson and Tait therefore developed a system which they

hoped would separate out their active material They applied the highly successful method of Zaffaroni and his collaborators (47 6), which consisted of fractionation on a propylene glycol toluene paper chromatographic system This method produces very good separation and also has a high capacity essential for handling extracts from large urine volumes They were able to elute the various fractions and test their ability to alter the urinary Na 24 / K 42 ratio The new compound was associated with the fraction containing cortisone By prolonged running of the extract in the same chromatographic system it was possible to separate the activity from cortisone and this disposed of the idea that their results could be explained by synergism of substances with cortisone While the fraction was free from cortisone it was obviously composed of a mixture of substances and further separations had to be effected The partition chromatography method of Bush (8), known as the Bush B5 paper system was used with great success and refinements in this technique led to the separation of the substance in a relatively pure form (45 21 36) Simpson and Tait suggested the name electrocortin for this material which they found to possess an activity certainly thirty to forty times if not more that of deoxycorticosterone Preliminary experiments made in the Middlesex Hospital Medical School seemed to indicate that the compound was of the same family as the known corticosteroids and that it probably possessed a δ-4-$\alpha\beta$ unsaturated ketone and an α ketol side chain In order to determine exactly the constitution large quantities of material were required and also the thorough knowledge of synthetic and analytical work in the subject of sterols Professor Reichstein became interested in this field as did also the laboratories of the Ciba Company and the Mayo Clinic As the result of combined efforts the actual constitution was published in a paper by the workers in Basel and the workers at the Middlesex Hospital Medical School (39) The constitution is shown in Figure 28 It will be seen that it can exist in two forms one with an oxygen bridge the other with an aldehyde group It is the 18 aldehyde of corticosterone and for this reason the name was changed to Aldosterone' by which it is now known universally It was announced last year by Wettstein (35) that the material had been synthesized but it is still not available commercially

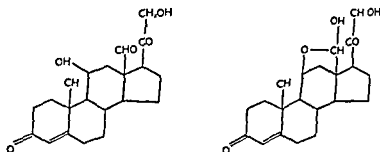


Figure 28 —Aldosterone

Biological properties of aldosterone

We have already referred to the suggested classification of adrenocortical steroids into mineralocorticoids and glucocorticoids according to whether their action is predominantly on mineral or carbohydrate metabolism Cortisone and hydrocortisone are the most potent steroids in relation to glucocorticoid metabolism whereas deoxycorticosterone, as mentioned was the most potent *as a mineralocorticoid although we must admit that this substance* had not until recently been detected as a constituent of the suprarenal cortex It was possible to apply quantitative methods to assess not only mineralocorticoid activity as we have already shown but also glucocorticoid activity If we compare the potency of aldosterone in relation to cortisone from a glucocorticoid point of view we find that it is very much less active than cortisone or hydrocortisone When however, we come to an investigation of the mineralocorticoid activity a number of tests have shown that aldosterone is some twenty five times more active than deoxy corticosterone in inducing sodium retention but apparently only five times more effective in producing potassium excretion (15 49) There appeared to be a number of differences between the actions of aldosterone and deoxycorticosterone For example, aldosterone is capable of producing sodium retention without associated water retention within certain limits This is unlike the action of deoxycorticosterone Swingle and his collaborators (42–44) have shown that aldosterone is not as effective in main

taining the blood pressure of adrenalectomized dogs as it is in maintaining the sodium level The first human experiments with aldosterone were performed by Mach and his collaborators (30 31) They showed the effect of intramuscular injection of the hormone in two patients with Addison's disease They maintained that aldosterone was twenty to thirty times more active than deoxy corticosterone in restoring the sodium and potassium balances and they also were the first to point out the rapid decrease in the pigmentation of the patients They drew attention to the fact that the glucose tolerance test returned to normal with a dose of 100–200 µg of aldosterone daily Similar results were obtained by Kekwick and Pawan (22), who showed that by administering 100 µg of aldosterone daily it is possible to maintain a well-estab lished Addison's disease satisfactorily Salassa and his collabo rators (34) treated three patients suffering from Addison s disease with aldosterone They noted gradual retention of sodium and water and frank clinical edema

The question of the secretion of aldosterone by the adrenal gland and its possible excretion in the urine was obviously a sub ject requiring investigation Although extremely accurate the sodium/potassium ratio method of estimating activity is very labo rious and even if the presence of highly active substances was confirmed in blood and urine it would still not be justifiable to assume that this was due to aldosterone In fact a number of workers have described the presence in human urine of highly active concentrations of mineralocorticoids Luetscher and his colleagues must receive the credit for the first clear-cut demonstra tion of aldosterone in the human urine They were able to obtain a crystalline substance from human urine and they proved con clusively that it was aldosterone (27–29) What is required is the estimation of hydrocortisone corticosterone and aldosterone in human urine under various conditions This has been partially accomplished by Luetscher and by Venning and their collaborators (46) who have shown that the ratio of aldosterone to hydrocorti sone secreted may alter in certain pathological conditions Re sults of this work and of Simpson and Tait seem to suggest that there is no relationship between the secretion of corticosterone hydrocortisone and aldosterone ACTH does not appear to alter

the excretion rate of aldosterone for a prolonged time, thus tending to indicate that its production by the suprarenal gland is apparently not under the direct control of the pituitary (1, 24, 26, 32)

Interesting as this observation is it indicates clearly the great difficulties in interpreting experimental results in this field especially if one considers that contemporary alterations in body fluids may modify aldosterone secretion It would obviously be possible, by altering the rate of secretion of hydrocortisone and corticosterone, to obtain alterations in the sodium/potassium ratio despite the fact that the aldosterone excertion rate remained constant Ayres, Garrod, Simpson, and Tait (2), at a recent meeting of the Royal Society of Medicine, reviewed the methods of estimating aldosterone in body fluids and proposed an entirely new method with which they have now had considerable experience The following is a description given at the meeting

The method now employed in our laboratory which is similar in principle to that of Farrell and his collaborators [17] and has also been used for preparing crystalline aldosterone consists essentially of the extensive purification of the diacetate followed by an objective measurement of the soda fluorescence

Concomitantly specific measurements of cortisol can be made Trace amounts of radioactive cortisol are added to one liter of a twenty four hour collection of urine, which is then continuously extracted with chloroform at room temperature at pH 1 for twenty four hours After further hand extraction and washing the chloroform extract is purified by silica gel chromatography (10) The dried residue from the ethyl acetate methanol fraction is chromatographed on a kieselguhr partition column (50) in which aldosterone and cortisol appear reproducibly together in the same effluent fractions An aliquot for bio assay for comparison purposes may be taken at this stage After acetylation and addition of 0 5 µg (^{14}C carboxyl) aldosterone diacetate these fractions are then chromatographed on another partition column in which the mobile phase is changed after the appearance of the aldosterone diacetate in the effluent Aliquots of the fractions are analyzed for radioactivity and those two containing most activity are paper chromatographed (8) The developed soda fluorescence is measured quantitatively by a fluorimeter arrangement (3) The specific activity of the steroids is thus obtained, and hence the amounts

initially present in the urine may be calculated A comparison of the results obtained for the measurement of aldosterone by bio assay and the physicochemical method for five normal urines gave good correlation Recoveries of free steroids throughout the pro cedure were about 70 per cent The coefficient of variation for duplicate determinations of aldosterone was 11 per cent

This method although probably of the required specificity sensitivity, and accuracy is very laborious It is tempting to be lieve that as a knowledge of the metabolites of the hormone is gained much easier methods will be evolved The urinary excre tion of tetrahydrocortisone plus tetrahydrocortisol in normal sub jects is about 6 mg/day (9) the secretion of cortisol being about 20 mg/day For the reasons previously presented the secretion of aldosterone is probably about 250 μg/day The maximal amount of the metabolites of aldosterone excreted in the urine would therefore be expected to be 250 μg/day, and that of any tetrahydro compound not more than 60 μg/day The only known measurable metabolite of aldosterone would appear to be the pre sumed conjugate which on acid hydrolysis yields 10 μg free aldosterone/day According to the above quantitative specula tions this should represent an appreciable fraction of the excre tion products of this hormone and it is doubtful whether the ana lytical procedure could be greatly simplified as a result of the char acterization of any tetrahydro derivatives of aldosterone unless a particularly specific reaction is developed

It is only by the use of such a method that the obscuring of the results by varying secretions of the other adrenocorticol steroids can be avoided There is little doubt that repetition of a number of observations would have to be made by the use of this admit tedly laborious method Despite the difficulty and unsatisfactory character of the methods for estimating aldosterone up to the pres ent some very interesting observations have emerged Conn (11–13) succeeded in grouping together a series of cases to which he gave the name of primary aldosteronism ' He described a series of symptoms associated with an increase in excretion of aldosterone in the urine and there now exists a body of literature to support this concept Primary aldosteronism is character ized by a series of symptoms in which very severe weakness and lethargy associated with tetany polyuria and hypertension are

included Edema and evidence or appearance of Cushing's syndrome are absent in these cases Laboratory findings show low blood potassium, high or normal blood sodium and an alkalosis as shown by an increase both in pH and in alkali reserve The excretion of 17 ketosteroids is quite normal Aldosterone as estimated in the urine is shown to be increased above normal Post mortem findings show characteristic lesions of the kidneys described as a clear cell nephrosis which is characteristic in cases of prolonged hypokalemia The syndrome may be associated with the presence of an adrenocortical adenoma, carcinoma or hyperplasia It is claimed that in some cases the condition is cured by their removal Conn has also considered the possible occurrence of a primary aldosteronopenia, a "nonrenal' salt losing syndrome Recently Ayres, Gould, Simpson, and Tait (4) have shown by direct evidence that aldosterone is preferentially produced in the glomerulosa and cortisol by the fasciculata This was already indicated previously by indirect evidence Probably the most interesting point about this work is that corticosterone is produced equally per gram weight by both zones Some of the metastases in a case of primary aldosteronism published by Foye and Feichtmeir (19) show clearly glomerulosa structure and Conn has reported high corticosterone values in the tumor of his case which was removed by operation This in vitro work in the laboratory would therefore perhaps give a clue to the explanation of these clinical findings Sufficient evidence has accumulated to show that aldosterone must play a very important part in diseases associated with altered electrolyte balance and may in fact prove to be of importance in many other hitherto unsuspected conditions

This extremely astute clinical application of scientific data at a very early stage has been confirmed and cases of primary aldosteronism are being described throughout the world

At the meeting of the Royal Society of Medicine already referred to, the clinical aspect of aldosterone excretion was reviewed The main object of this meeting was to discuss factors concerning the regulation of the excretion of aldosterone It was pointed out that while Singer and Stacke Dunne (40) had reported great reduction of corticosterone in hypophysectomized rats aldosterone secretion was not much affected Similar results have been ob

tained in hypophysectomized dogs In man Luetscher and Axelrad (26) reported that the aldosterone secretion in two patients with severe hypopituitary symptoms was not appreciably affected The administration of large doses of ACTH did not affect the urinary excretion of aldosterone An alternative method of approaching this problem is to suppress the endogenous production of cortico trophin by giving large amounts of cortisone This has been done by Farrell and his collaborators in the dog They showed that after five weeks of 100 mg of cortisone daily the secretion of cortisol and cortisone measured in the adrenal vein fell to almost disappear ing point while the aldosterone secretion was unaffected It has also been shown by Liddle and his collaborators (23) that the aldosterone excretion rate in man cannot be affected by large doses of cortisone Prunty and his collaborators (33) performed the reverse experiment and reported that large doses of aldosterone given to a patient with adrenogenital syndrome did not suppress the excretion of the excess of 17 ketosteroids These observations together with those already summarized would indicate quite clearly that the excretion of aldosterone is independent of pituitary control The point naturally arises as to what influences the rate of secretion of aldosterone The known facts can be summarized under the following headings

1 *Salt depletion* —It has been shown by Luetscher and Axel rad (25) that if the sodium excretion is diminished by sodium deprivation the aldosterone content of the urine rises by some five times

2 *Sweating* —Falbriard and his collaborators (16) showed that loss of water due to excessive sweating was attended by a rapid rise in aldosterone excretion

3 *Hemorrhage* —Farrell and his collaborators (17) have shown that in the dog hemorrhage is followed by a marked increase in aldosterone excretion All the above conditions cause an in crease in aldosterone excretion

A decrease or suppression of secretion can be obtained in the following ways

1 *Sodium loading* —Garrod (20) showed that by increasing the salt content of a normal subject by 20 gm for two days the aldosterone excretion fell to nearly one third of the level of the

control Forty-eight hours after discontinuing the additional salt the aldosterone excretion returned to normal

2 *Severe potassium depletion*

3 *Excessive water retention*

The two latter conditions can give rise to a fall in the aldosterone excretion

The literature is now beginning to contain a number of references to aldosteronism, and it would appear that this condition can now explain an association of symptoms hitherto quite inexplicable A good example of this can be found in the recent paper by Crane, Vogel, and Richland (14) A patient, an Italian man of thirty two years who was referred to the neurosurgical service because of persistent hypertension complained of excessive dryness of the throat To combat this he drank two to three liters of water daily, but obtained only transitory relief It was for this complaint he originally came to hospital and the hypertension was found as a result of routine examination An extensive course of Rauwolfia drugs and a low sodium diet failed to relieve either the dryness of the mouth or the hypertension A very elaborate questioning of the patient revealed that during the past five months he had had sporadic episodes of weakness in his arms and legs, lasting from hours to days and on one occasion this weakness was so severe as to keep him from work A detailed investigation of the patient showed that in addition to the original physical findings he had alkalosis and hypokalemia On the basis of this it was suggested that the patient had an adrenal tumor or hyperfunctioning adrenal cortex with the overproduction of aldosterone Unfortunately these workers were not able to estimate the hormone Their confidence however was sufficiently great to make a diagnosis The patient was operated on and a small benign adenoma was found This was removed and the patient made an uneventful recovery

Here we can see that until the presence of aldosterone was demonstrated it would have been impossible to explain a case of this type

Recently it has been possible to demonstrate the site of origin of aldosterone

Farrell, Banks and Koletsky (18) produced evidence based on histological examination of adrenal venous blood that aldo

sterone is probably produced by the *zona glomerulosa* and cortisol by the *zona fasciculata* Ayres Gould, Simpson, and Tait (4), in a recent paper, have confirmed these results, using slices of ox adrenal cortex

It was also interesting to note that these results did not apply to the production of corticosterone in the ox gland

While it is impossible at the present time to give any certain clue to the factors controlling aldosterone excretion, it appears to be mainly bodily requirements for fluid volume control

It is difficult to predicate what the use of aldosterone would be if it was available in large quantities One of the most important points to decide is whether the material differs qualitatively as well as quantitatively from the action of deoxycorticosterone If the difference is merely a quantitative one, then obviously it would be much easier to give a little more deoxycorticosterone than less of the much more expensive aldosterone The experiments on Addison s disease already mentioned would seem to indicate that there is a qualitative difference and until a large amount is available for clinical trial an answer to this problem cannot be given It is interesting that both stilbestrol and aldosterone immediately attracted the attention of workers throughout the world and that, despite the fact that aldosterone and electrocortin have only been known a very few years they have already begun to play an important part in pathological and medical considerations

In a recent symposium Bartter (5) reviewed the role of aldosterone in normal homeostasis and in certain diseased states This author reaffirms that aldosterone exerts its effect on sodium potassium and hydrogen ions by altering the renal tubular mechanism He supports the view that the secretion of aldosterone is controlled by the extracellular body fluid volume but he also feels that the potassium ions and to a lesser extent ACTH content of the extracellular body fluid may have an effect The urinary excretion of aldosterone is increased in cirrhosis of the liver nephrosis and cardiac failure He suggests that a study of the urinary sodium excretion when the patient is on a very low sodium diet is a valuable investigation to differentiate between the hyperaldosteronism associated with the diseases mentioned above and that associated with adrenal tumor or hyperplasia

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It was also interesting to note that these results did not apply to the production of corticosterone in the ox gland

While it is impossible at the present time to give any certain clue to the factors controlling aldosterone excretion, it appears to be mainly bodily requirements for fluid volume control

It is difficult to predicate what the use of aldosterone would be if it was available in large quantities One of the most important points to decide is whether the material differs qualitatively as well as quantitatively from the action of deoxycorticosterone If the difference is merely a quantitative one, then obviously it would be much easier to give a little more deoxycorticosterone than less of the much more expensive aldosterone The experiments on Addison s disease, already mentioned would seem to indicate that there is a qualitative difference and until a large amount is available for clinical trial an answer to this problem cannot be given It is interesting that both stilbestrol and aldosterone immediately attracted the attention of workers throughout the world and that despite the fact that aldosterone and electrocortin have only been known a very few years they have already begun to play an important part in pathological and medical considerations

In a recent symposium Bartter (5) reviewed the role of aldosterone in normal homeostasis and in certain diseased states This author reaffirms that aldosterone exerts its effect on sodium potassium and hydrogen ions by altering the renal tubular mechanism He supports the view that the secretion of aldosterone is controlled by the extracellular body fluid volume but he also feels that the potassium ions and to a lesser extent ACTH content of the extracellular body fluid may have an effect The urinary excretion of aldosterone is increased in cirrhosis of the liver nephrosis and cardiac failure He suggests that a study of the urinary sodium excretion when the patient is on a very low sodium diet is a valuable investigation to differentiate between the hyperaldosteronism associated with the diseases mentioned above and that associated with adrenal tumor or hyperplasia

The investigations recounted in these five lectures bear out the old view that research should be pursued for itself and not with any immediate practical object in mind No one, for example, could have foreseen that the early experiments with synthetic estrogens in changing the plumage of capons were likely to lead to a substance having a powerful influence on one form of cancer while the investigation of an outmoded suprarenal extract did not seem a very promising start for the investigation that led eventually to the isolation and characterization of an entirely new suprarenal principle

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